MANUAL OF METHODS OF ANALYSIS OF FOODS

SWEETS & CONFECTIONARY INCLUDING SWEETENING AGENT

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Note: The test methods given in the manual are standardized / validated and were taken from national or international methods or recognized specifications, however it would be the responsibility of the respective testing laboratory to verify the performance of these methods onsite and ensure that it gives proper results before putting these methods in to use.

Part A: SWEETS & CONFECTIONARY

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Method No.	FSSAI 04C.001:2024	**Revision No. & Date**	0.0
Scope	Cocoa Powder, Dry Mixtures of cocoa and sugars, Cocoa mass or cocoa/chocolate Liquor and cocoa cake		
Caution	Once sample is opened, seal it in airtight manner after taking test portion		
Principle	Moisture is the weight lost due to evaporation of water present in a sample. The sample is dried under controlled conditions to remove moisture during the analysis. To determine moisture content, the difference in sample weight before and after drying is calculated.		
Apparatus/Instrument	General Apparatus and Glassware 1. Platinum / Stainless steel dish/Aluminium dish 2. Hot Air oven 3. Weighing balance (Accuracy of weighing balance should be ±0.001 g) 4. Dessicator		
Materials and Reagents	1. Dessicants		
Sample Preparation	Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, after withdrawal of test portions for analytical determinations.		
Method of analysis	1. Weigh accurately about 2 g of sample in Platinum / stainless steel dish. 2. Distribute the material as evenly as possible and place in a hot air oven maintained at 100 °C (in case of Cocoa mass or cocoa/chocolate Liquor and cocoa cake 105 ± 2 °C). Dry to a constant weight (aluminium dish may be used when ash is not determined on the same sample). 3. Repeat the operation until the difference between two successive weighing is less than 1mg. Record the lowest mass. 4. Report loss in weight as moisture.		
Calculation with units of expression	Moisture (%) (by weight) = $\frac{W_1 - W_2}{W1 - W} \times 100$ Where, W = Weight of empty dish		

	W_1 = Weight of dish + sample before drying W_2 = Weight of dish + dried sample
Inference (Qualitative Analysis)	NA
Reference	A.O.A.C 21st edn, Official Method of Analysis (2019) Method no.931.04 Moisture in Cocoa Products. IS :1164-1986 Specification for Cocoa Powder IS :11923 -:2022 Cocoa Mass (Cocoa/Chocolate Liquor) — Specification
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.002:2024	**Revision No. & Date**	0.0
Scope	Sugar boiled confectionery & Lozenges, Chewing gum and bubble gum		
Caution	Once sample is opened, seal it in airtight manner after taking test portion		
Principle	Sample is heated in a vacuum oven under controlled conditions of pressure and temperature to remove moisture by passing dry air. Sample is weighed before and after drying and the difference in sample weight before and after drying is calculated.		
Apparatus/Instrument	General Apparatus and Glassware 1. Aluminum dish – 75 mm diameter and about 25 mm height with close fitting cover 2. Desiccator 3. Vacuum oven 4. Weighing balance (Accuracy of weighing balance should be ±0.001 g)		
Materials and Reagents	Desiccants for Desiccator		
Sample Preparation	**Sugar boiled confectionery & Lozenges** If composition of entire product is desired, grind and mix thoroughly. If product is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as completely as possible, and grind and mix each test portion thoroughly. **Chewing gum and bubble gum** Cut into small bits/ pieces around 50-75 g and mix well. Stored in an airtight container.		
Method of analysis	1. Accurately weigh about 5 g of sample, in a flat dish with tight-fit cover having a diameter of about 75 mm and a height of about 25 mm previously dried and weighed. Distribute the material as evenly as possible over the bottom of the dish by gentle sidewise movements. 2. Place dish in vacuum oven, remove cover of dish and dry the material for 2 h at 70 ± 1 °C at a pressure not exceeding 50 mm of Hg. During heating admit slow current of air into oven. 3. Cover dish, transfer to desiccator and weigh soon after room temperature is attained. 4. Re-dry for 1 h and repeat the process till the difference between the two successive weighing is less than 2 mg. Report percent loss in weight as moisture %.		

Calculation with units of expression	Moisture (%) = $\frac{W_1 - W_2}{W1 - W} \times 100$ (by weight) Where, W = Weight in g, of empty Aluminium dish. W_1 = Weight in g, of Aluminium dish + sample before drying. W_2 = Weight in g, of Aluminium dish + dried sample.
Inference (Qualitative Analysis)	NA
Reference	IS: 6287-1985 (Reaffirmed 2020) Methods of Sampling and Analysis for Sugar Confectionery IS: 6287-2002 Methods of Sampling and Analysis for Sugar Confectionery
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Method No.	FSSAI 04C.003:2024	**Revision No. & Date**	0.0
Scope	Cocoa mass or cocoa/chocolate Liquor and cocoa cake, Dry Mixtures of cocoa and sugars		
Caution	Once sample is opened, seal it in airtight manner after taking test portion		
Principle	Moisture is the weight lost due to evaporation of water present in a sample. The Cocoa sample is dried under controlled conditions to remove moisture during the analysis. To determine moisture content, the difference in sample weight before and after drying is calculated.		
Apparatus/Instrument	General Apparatus and Glassware 1. Weighing bottle(Diameter of about 40 mm and a height of about 25 mm) 2. Vacuum oven 3. Weighing balance (Accuracy of weighing balance should be ±0.001 g) 4. Dessicator		
Materials and Reagents	Dessicants		
Sample Preparation	Cool the material until hard and then grate or shear to a fine granular condition.		
Method of analysis	1. Weigh accurately about 10 g of the prepared sample in a tared weighing bottle having a diameter of about 40 mm and a height of about 25 mm. 2. Distribute the material as evenly as possible over the bottom of the bottle by gentle tapping. 3. Place the bottle in a vaccum oven, remove the cover of the bottle and dry the material for 6 h at 80 ± 1 °C at a pressure not exceeding 5 mm of mercury. 4. Allow the bottle to cool to room temperature and weigh.		
Calculation with units of expression	Moisture (%) (by weight) $= \frac{W1 - W2}{W1 - W} \times 100$ Where, W = Weight of empty dish. W1 = Weight of dish + sample before drying.		

	W2 = Weight of dish + dried sample.
Inference (Qualitative Analysis)	NA
Reference	IS : 1164 -1986 Cocoa Powder IS :11923|- 2022 Cocoa Mass (Cocoa/Chocolate Liquor) — Specification
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.004:2024	**Revision No. & Date**	0.0
Scope	Sugar Boiled Confectionery & Lozenges, Chewing gum and bubble gum		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Concentrated hydrochloric acid is corrosive, has an irritant vapour and causes burns. Wear mask and gloves during analysis		
Principle	Total ash is dissolved in dilute hydrochloric acid and acid in-soluble ash is determined. The proportion of ash that is not hydrolyzed by acid is known as the acid insoluble ash (silica and oxalates). The sample is ashed at a temperature 550°C ± 25 and the residue weighed.		
Apparatus/Instrument	General Apparatus and Glassware 1. Weighing Balance. 2. Platinum dish – 100 mL capacity. 3. Burner. 4. Muffle furnace.		
Materials and Reagents	1. Concentrated hydrochloric acid. 2. Silver nitrate. 3. Nitric acid.		
Preparation of Reagents	1. Dilute Hydrochloric acid (Approx 5 N): Hydrochloric acid (445 mL) diluted to 1 L using distilled water.		
Sample Preparation	**Sugar boiled confectionery & Lozenges** If composition of entire product is desired, grind and mix thoroughly. If product is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as completely as possible, and grind and mix each test portion thoroughly. **Chewing gum and bubble gum** Cut into small bits/ pieces around 50-75 g and mix well. Stored in an airtight container. **Chocolate** 1. Melt the product in a beaker at a temperature of 45-50 °C. Pour the melted sample on a marble slab and mix thoroughly with a spatula till the product is solidified and transfer to a stoppered glass bottle. Store in a cool place. 2. Chill the material until hard and then grate or shear to a fine granular		

	condition. Mix thoroughly and transfer to a stoppered glass bottle. Store in a cool place. 3. Alternatively melt in a suitable container by placing container in water bath at about 50 °C. Stir frequently until test portion melts and reaches temperature of 45 – 50 °C, remove from bath, stir thoroughly and while still hot remove test portion for analysis using glass or metallic tube provided with close fitting plunger to expel test portion from tube or disposable plastic syringe.
Method of analysis	1. Weigh accurately about 5 g of the prepared sample in a tared, clean and dry platinum dish of 100 mL capacity. 2. Carbonize the material in the dish with the flame of a burner. 3. Complete the ignition by keeping in a muffle furnace at 550 $\pm$ 25 °C until gray ash results. 4. Cool in a desiccator. 5. To the ash, add 25 mL of the dilute hydrochloric acid, cover with a watch glass and heat on a small flame of a burner to near boiling. 6. Allow it to cool and filter the contents of dish through Whatman filter paper No. 42 or its equivalent. Wash the filter paper and residue with hot water until the washings are free from chlorides (To check this, add few drops of 2 M Nitric acid and 0.1 M Silver nitrate solution to the filtrate obtained. No precipitate or milky turbidity should occur in the solution, if it is chloride-free.) 7. Return the filter paper and the residue to the dish. Keep it in an air oven maintained at 105 $\pm$ 2 °C for about 3 h. Ignite in the muffle furnace at 550 $\pm$ 25 °C for 1 h. 8. Cool the dish in a desiccator and weigh. 9. Heat again for 30 min in the muffle furnace, cool and weigh. 10. Repeat this process of heating for 30 min, cooling and weighing till the difference between two successive weighing is less than one milligram. Note the lowest mass.
Calculation with units of expression	Acid insoluble ash,% by mass = $\frac{M1 \times 100}{M2}$ Where,

	M1 = mass in g of the acid insoluble ash M2 = mass in g of the prepared sample taken for the test
Inference (Qualitative Analysis)	NA
Reference	IS: 6287-1985 Methods of Sampling and Analysis for Sugar Confectionery
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.005:2024	**Revision No. & Date**	0.0
Scope	Cocoa mass or cocoa/chocolate Liquor and cocoa cake, Chocolate and Cocoa Powder		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Concentrated hydrochloric acid is corrosive, has an irritant vapour and causes burns. Wear mask and gloves during analysis		
Principle	Total ash is dissolved in dilute hydrochloric acid and acid in-soluble ash is determined. The proportion of ash that is not hydrolyzed by acid is known as the acid insoluble ash (silica and oxalates). The sample is ashed at a temperature 550°C ± 25 and the residue weighed.		
Apparatus/Instrument	General Apparatus and Glassware 1. Silica dish 2. Muffle furnace 3. Burner 4. Filter paper - Ash less (Whatman 41 or 42 or equivalent) 5. Filtration system		
Materials and Reagents	1. Conc. Hydrochloric acid 2. Distilled Water		
Preparation of Reagents	1. **Dilute Hydrochloric Acid**, approximately 5 N, prepared from concentrated hydrochloric acid		
Sample Preparation	Cool the material until hard and then grate or shear to a fine granular condition.		
Method of analysis	1. Weigh accurately about 10 g of the material in a porcelain dish. 2. Heat at 100 °C until water is expelled and then heat slowly over a flame until swelling ceased. Ignite in a muffle furnace at 550 °C until grey ash results. 3. To the ash contained in the dish, add 25 ml of dilute hydrochloric acid, cover with a watch glass and heat on a boiling water bath for 10 min.. 4. Allow to cool and filter the contents of the dish through Whatman filter paper No. 42 or its equivalent. Wash the filter paper until the washings are free from the acid.		

	5. Return the filter paper and residue to the dish. Keep it in an electric air oven maintained at 135 + 2 °C for about 3 h. 6. Cool the dish in a desiccator and weigh. Repeat the process of igniting in a muffle furnace, cooling and weighing on half hour intervals until the difference in mass between two successive weighings is less than 1 mg. Note the lowest mass.
Calculation with units of expression	Ash insoluble in dilute HCl (%) (on dry wt.) = $\frac{m_1 \times 100 \times 100}{m_2 \times (100 - (M+F))}$ where, m_1 = mass in g, of the acid insoluble ash; m_2 = mass in g, of the prepared sample taken for the test; M = moisture, percent by mass in the prepared sample and F = fat (on as is basis), percent by mass, in the prepared sample.
Inference (Qualitative Analysis)	NA
Reference	IS-11923|:2022 Cocoa Mass (Cocoa/Chocolate Liquor) — Specification IS: 1163-2011 Specification for chocolates.
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.006:2024	**Revision No. & Date**	0.0
Scope	Sugar Boiled Confectionery & Lozenges, Chewing Gum And Bubble Gum		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Concentrated sulphuric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Sulphated ash test utilizes a procedure to measure the amount of residual substance not volatilized from organic sample when the sample is ignited in the presence of sulphuric acid. The sample is ashed at a temperature 550°C ± 25 and the residue weighed.		
Apparatus/Instrument	General Apparatus and Glassware 1. Weighing balance. 2. Platinum dish (dia. 9cm). 3. Hot plate. 4. Muffle furnace. 5. Desiccator. 6. Exhaust hood.		
Materials and Reagents	1. Concentrated Sulphuric Acid. 2. Desiccants for Desiccator.		
Preparation of Reagents	1. Sulphuric Acid - 10 %(m/v)		
Sample Preparation	**Sugar boiled confectionery & Lozenges** If composition of entire product is desired, grind and mix thoroughly. If product is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as completely as possible, and grind and mix each test portion thoroughly. **Chewing gum and bubble gum** Cut into small bits/ pieces around 50-75 g and mix well. Stored in an airtight container.		
Method of analysis	1. Accurately weigh about 5 g of the prepared sample into a 9 cm diameter platinum basin. 2. Add 5 mL of sulphuric acid to the material in the dish. Gently heat the dish on a hot plate (in exhaust hood) until the material is well carbonized and then increase the heat until the evolution of sulphuric acid fumes ceases. 3. Ash the carbonized matter in a muffle furnace at 550 ± 25 ^{o}C. 4. Cool the ash and moisten it with 2-3 mL of sulphuric acid.		

	5. Heat strongly on a hot plate until sulphuric acid fumes ceases to be evolved and finally ash in the muffle furnace at 550 ± 25 ^{o}C for 2 h. 6. Cool in a desiccator and weigh. 7. Heat again in a muffle furnace for 30 min at 550 ± 25 ^{o}C. Cool in a desiccator and weigh. 8. Repeat the process of heating in the muffle furnace for 30 min, cooling and weighing till the difference between two successive weighing is less than 1 mg. Record the lowest mass.
Calculation with units of expression	Sulphated ash, % by mass = $\frac{M1 \times 100}{M2}$ Where, M1 = mass in g of the sulphated ash M2 = mass in g of the prepared sample taken for the test.
Inference (Qualitative Analysis)	NA
Reference	IS: 6287-1985 Methods of Sampling and Analysis for Sugar Confectionery.
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.007:2024	**Revision No. & Date**	0.0
Scope	Cocoa powder**,** Cocoa mass or cocoa/chocolate Liquor and cocoa cake		
Caution	Concentrated Hydrochloric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Alkalinity of ash is a measure of presence of combined cations with organic acids and indicates its conductivity. Alkalinity of water soluble ash was determined using hydrochloric acid.		
Apparatus/Instrument	General Apparatus and Glassware 1. Weighing Balance 2. porcelain dish 3. muffle furnace		
Materials and Reagents	1. Dilute Hydrochloric Acid - approximately 0-1 N 2. Standard Sodium Hydroxide- approximately 0·1 N, accurately standardized		
Sample Preparation	Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, after withdrawal of test portions for analytical determinations.		
Method of analysis	1. Weigh accurately about 2 g of the material. 2. Heat at 100°C until water is expelled and then heat slowly over a flame until swelling ceases. 3. Ignite in a muffle furnace at 550°C until grey ash results. 4. Add a known excess of dilute hydrochloric acid and boil for 2 minutes. 5. Cool and titrate the excess of acid against standard sodium hydroxide using bromocresol green as indicator, till the colour changes to green. 6. Titrate 10 ml of dilute hydrochloric acid against the standard sodium hydroxide using phenolphthalein as indicator, till the colour changes to pink.		
Calculation with units of expression	Alkalinity of ash (as KaO) (on moisture and fat free basis), percent by $\text{mass} = \frac{47.1*N\,(V2V1-V3)}{10} \Big/ m[100-(M+F)]$		

	where N = normality of standard sodium hydroxide V2 — volume in ml of dilute hydrochloric acid added V1 = volume in ml of standard sodium hydroxide corresponding to 10 ml of dilute hydrochloric acid V3 = volume in ml of standard sodium hydroxide required for the excess of acid m = mass in g of the material taken for the test M = moisture, percent by mass, in the material F = fat (cocoa-butter), percent by mass, in the material
Inference (Qualitative Analysis)	NA
Reference	IS : 1164 -1986 Cocoa Powder IS :11923 - 2022 Cocoa Mass (Cocoa/Chocolate Liquor) — Specification
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.008:2024	**Revision No. & Date**	0.0
Scope	Sugar Boiled Confectionery & Lozenges, Chewing gum and bubble gum, Ice lollies or edible ices		
Caution	Concentrated Hydrochloric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Sample (Sucrose) is inverted using acid and neutralized solution is titrated against Fehling solution. Subtraction of the reducing sugars provides the sucrose content		
Apparatus/Instrument	General Apparatus and Glassware 1. Water bath with heating system. 2. Weighing balance. 3. Volumetric flask. 4. Burette 5. Pipette 6. Conical flasks 7. Burner 8. Wire guage		
Materials and Reagents	1. Hydrochloric acid 2. Sodium hydroxide 3. Sodium carbonate 4. Copper sulphate 5. Potassium sodium tartrate 6. Methylene blue		
Preparation of Reagents	1. Fehling A: Dissolve 69.28 g copper sulphate ($CuSO_4.5H_2O$) in distilled water. Dilute to 1000 mL. Add 0.5ml of Concentrated H_2SO_4 in it. Filter the mixture and store in amber coloured bottle. 2. Fehling B: Dissolve 346 g Rochelle salt (Potassium Sodium tartrate) (K Na $C_4H_4O_6$. $4H_2O$) and 100 g NaOH in distilled water. Dilute to 1000 mL. Filter and store in amber coloured bottle. 3. Carrez 1 – Add 21.9 g Zinc acetate and 3 mL acetic acid in a 100 mL volumetric flask. Make up the volume with water. 4. Carrez 2 – 10.6% aqueous solution of Potassium ferrocyanide. 5. Methylene Blue Indicator: Prepare 1% of methylene blue solution in distilled water.		
Sample Preparation	**Sugar boiled confectionery & Lozenges** If composition of entire product is desired, grind and mix thoroughly. If product is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as completely as possible, and grind and mix each test portion thoroughly.		

	Chewing gum and bubble gum Cut into small bits/ pieces around 50-75 g and mix well. Stored in an airtight container.
Method of analysis	1. Take an aliquot of the filtrate obtained in reducing sugar method and invert it with Hydrochloric acid in a water bath at 60 °C by keeping for 10 min. 2. Cool immediately and neutralize with sodium hydroxide and finally with sodium carbonate. Make up to volume and determine reducing sugar as above. **Determination of Factor (for Invert Sugar) of Fehling Solution:** 3. Accurately weigh around 4.75 g of sucrose. 4. Transfer to 500 mL volume flask with 50 mL distilled water. 5. Add 5 mL conc. HCl and allow to stand for 24 h. 6. Neutralize with NaOH solution and make up to volume. 7. Mix well and transfer 50 mL to a 100 mL volumetric flask and make up to volume. 8. Transfer to a burette having an offset tip. 9. Perform the titration of Fehling solution following the similar procedure as above: $\text{Fehling Factor (as Invert Sugar)} = \frac{\text{Titre x Weight of sucrose in g}}{500}$
Calculation with units of expression	$\text{Total Reducing Sugars \% (as Invert Sugar)} = \frac{\text{Dilution x Factor of Fehling solution (in g)}}{\text{Weight of sample x Titre value}}$ Sucrose % = (Total reducing sugars / invert sugar % - reducing sugars %) x 0.95
Inference (Qualitative Analysis)	NA
Reference	IS: 6287- 1985 Methods of Sampling and Analysis for Sugar Confectionery
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.009:2024	**Revision No. & Date**	0.0
Scope	Sugar Boiled Confectionery & Lozenges, Chewing gum and Bubble gum		
Caution	Wear mask and gloves during analysis		
Principle	Invert sugar reduces the copper in Fehling's solution to red, insoluble cuprous oxide. The sugar content in a food sample is estimated by determining the volume of the unknown sugar solution required to completely reduce a known volume of Fehling's solution. Glucose and other sugars are capable of reducing oxidizing agents and are called reducing sugars		
Apparatus/Instrument	General Apparatus and Glassware 1. Weighing balance. 2. Volumetric flask. 3. Amber coloured bottles. 4. Filter paper. 5. Pipettes – 5 mL 6. Burettes. 7. Conical flasks -250 mL 8. Dropper 9. Glass beads / fume stones 10. Burner.		
Materials and Reagents	(1) Copper sulphate ($CuSO_4.5H_2O$). (2) Distilled water. (3) Rochelle salt (Potassium Sodiumtartrate) (K Na $C_4H_4O_6$. $4H_2O$). (4) Sodium hydroxide. (5) Zinc acetate. (6) Acetic acid. (7) Potassium ferrocyanide. (8) Methylene Blue Indicator. (9) Dextrose anhydrous		
Preparation of Reagents	1. Fehling A: Dissolve 69.28 g coppersulphate ($CuSO_4.5H_2O$) in distilled water. Dilute to 1000 mL. Filter and store in amber coloured bottle. 2. Fehling B: Dissolve 346 g Rochelle salt (Potassium Sodiumtartrate) (K Na $C_4H_4O_6$. $4H_2O$) and 100 g NaOH in distilled water. Dilute to 1000 mL. Filter and store in amber coloured bottle. 3. Carrez 1 – Add 21.9 g Zinc acetate and 3 mL acetic acid in a 100 mL volumetric flask. Make up the volume with water. 4. Carrez 2 – 10.6% aqueous solution of Potassium ferrocyanide. 5. Methylene Blue Indicator: Prepare 1% of methyleneblue solution in		

	distilled water.
Sample Preparation	**Sugar boiled confectionery & Lozenges** If composition of entire product is desired, grind and mix thoroughly. If product is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as completely as possible, and grind and mix each test portion thoroughly. **Chewing gum and bubble gum** Cut into small bits/ pieces around 50-75 g and mix well. Stored in an airtight container.
Method of analysis	1. Weigh accurately about 5 g sample, transfer to a 200 mL volumetric flask dissolve in warm water, dilute to about 150 mL. 2. In case solution is not clear, add 5 mL of Carrez 1 solution followed by 5mL of Carrez 2 solution. 3. Make up to 200 mL. Filter through a dry filter paper. 4. Titrate the solution obtained as such to determine % Reducing sugars. **Preliminary Titration:** **5.** Pipet 5 mL each of Fehling A and B into 250 mL conical flask. Mix and add about 10 mL water and a few boiling chips or glass beads. **6.** Dispense solution. Heat the flask to boiling. **7.** Add 3 drops of methyleneblue indicator. **8.** Continue the addition of solution drop wise until the blue colour disappears to a brick-red end point. (The concentration of the sample solution should be such that the titre value is between 15 and 50 mL). **9.** Note down the titre value. **Final Titration:** 10. Pipet 5 mL each of Fehling A and B. 11. Add sample solution about 2 mL less than titre value of the preliminary titration. 12. Heat the flask to boiling within 3 min and complete the titration. 13. Perform the titration duplicate and take the average. **Determination of Factor of Fehling Solution:** 14. Accurately weigh known quantity of analytical grade glucose around 4.5 g. 15. Transfer to 500 mL volume flask with 50 mL distilled water and make up to volume. 16. Mix well and transfer 50 mL to a 100 mL volumetric flask and make up to volume. 17. Transfer to a burette having an offset tip. 18. Perform the titration of Fehling solution following the similar procedure as above:

	Fehling Factor = $\frac{\text{Titre x Weight of glucose in g}}{500}$
Calculation with units of expression	Calculate the reducing sugar % as shown below. Reducing Sugars % = $\frac{\text{Dilution x Factor of Fehling solution (in g)}}{\text{Weight of sample x Titre value}}$
Inference (Qualitative Analysis)	NA
Reference	IS: 6287-1985 Methods of Sampling and Analysis for Sugar Confectioner
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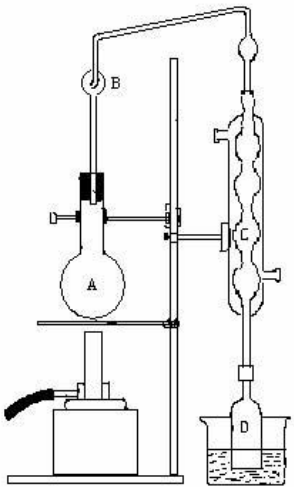
Method No.	FSSAI 04C.010:2024	**Revision No. & Date**	0.0
Scope	Sugar Boiled Confectionery & Lozenges, Chewing Gum And Bubble Gum		
Caution	Concentrated Sulphuric acid is corrosive, causes burns. Wear mask and gloves during analysis.		
Principle	Organically bonded nitrogen is converted into ammonium ions in digestion process which is further converted into ammonia during distillation and concentration of ammonia determined by acid base titration.		
Apparatus/Instrument	General Apparatus and Glassware 1. A recommended distillation assembly is shown below - The assembly consists of a round bottom flask A of 1000 mL capacity fitted with a rubber stopper through which passes one end of the connecting bulb tube B. the other end of the bulb B is connected to the condenser C which is attached, by means of a rubber tube, to a dip tube D which dips into a known quantity of standard sulphuric acid contained in a beaker of 250 mL capacity.  *Distillation assembly for Protein Estimation* (1) Kjeldahl flask – 500 mL capacity. (2) Weighing balance. (3) Burner. (4) Round bottom flask.		
Materials and Reagents	(1) Anhydrous Sodium sulphate (2) Copper sulphate		

	(3) Concentrated Sulphuric acid- sp gr 1.84 (4) Sodium hydroxide (5) Standard sulphuric acid (6) Methyl red indicator.
Preparation of Reagents	(1) Sodium hydroxide solution- Dissolve about 225 g of sodium hydroxide in 500 mL of water (2) Standard Sulphuric acid- 0.1 N (3) Methyl red indicator solution- Dissolve 1g of methyl red in 200mL of Rectified spirit (95 %v/v) (4) Standard sodium hydroxide solution -0.1 N *Boric acid can also be used instead of sulphuric acid.
Sample Preparation	**Sugar boiled confectionery & Lozenges** If composition of entire product is desired, grind and mix thoroughly. If product is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as completely as possible, and grind and mix each test portion thoroughly. **Chewing gum and bubble gum** Cut into small bits/ pieces around 50-75 g and mix well. Stored in an airtight container. **Chocolate** 1. Melt the product in a beaker at a temperature of 45-50 °C. Pour the melted sample on a marble slab and mix thoroughly with a spatula till the product is solidified and transfer to a stoppered glass bottle. Store in a cool place. 2. Chill the material until hard and then grate or shear to a fine granular condition. Mix thoroughly and transfer to a stoppered glass bottle. Store in a cool place. 3. Alternatively melt in a suitable container by placing container in water bath at about 50 °C. Stir frequently until test portion melts and reaches temperature of 45 – 50 °C, remove from bath, stir thoroughly and while still hot remove test portion for analysis using glass or metallic tube provided with close fitting plunger to expel test portion from tube or disposable plastic syringe.

Method of analysis	1. Transfer carefully about 1 or 2 grams of the sample accurately weighed, to the Kjeldhal flask, taking precaution to see that particles of the material do not stick to the neck of the flask. 2. Add about 10 g of anhydrous sodium sulphate, 0.2 to 0.3 g of copper sulphate and 20 mL of concentrated sulphuric acid. 3. Place the flask in an inclined position. Heat below the boiling point of the acid until frothing ceases. Increase heat until the acid boils vigorously and digests for 30 min after the mixture becomes clear and pale green in colour. Cool the flask. 4. Transfer quantitatively to the round-bottomed flask with water, the total quantity of water used being about 200 mL. Add a few pieces of pumice stones to avoid bumping. Add about 50 mL of Sodium hydroxide solution (which is sufficient to make the solution alkaline) carefully through the side of the flask so that it does not mix with the acid solution but forms a separate layer below the acid layer. 5. Assemble the apparatus as shown above taking care that the dip tube extends below the surface of the standard sulphuric acid solution contained in the beaker. 6. Mix the contents of the flask by shaking and distil until all the ammonia has passed over into the standard sulphuric acid. 7. Shut off the burner and immediately detach the flask from the condenser. Rinse the condenser thoroughly with water into the beaker. Wash the dip tube carefully so that all traces of the condensate are transferred to the beaker. 8. When all the washings have been drained into the beaker, add two or three drops of methyl red indicator solution and titrate with the standard sodium hydroxide solution. 9. Carry out a blank determination using all reagents in the same quantities but without the sample to be tested.
Calculation with units of expression	Total Protein (N x 6.25), % by mass= $\frac{8.75 \times (B-A) \times N}{M}$ Where, B = volume in mL of the standard sodium hydroxide solution used to neutralize the acid in the blank determination A = volume in mL of the standard sodium hydroxide solution used to neutralize the excess of the acid in the test with the material N = Normality of the standard sodium hydroxide solution M = mass in g of the material taken for the test
Inference (Qualitative Analysis)	NA
Reference	1. IS: 6287-1985 Methods of Sampling and Analysis for Sugar

	Confectionery.
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Method No.	FSSAI 04C.011:2024	**Revision No. & Date**	0.0
Scope	Sugar Boiled Confectionery & Lozenges, Chewing Gum And Bubble Gum		
Caution	Wear mask and gloves during analysis while handling solvents		
Principle	Sample is treated with hot water and fat is extracted with diethyl ether and petroleum ether. Mixed ethers are evaporated and the residue weighed.		
Apparatus/Instrument	General Apparatus and Glassware (Page 3 and 4) (1) Mojonnier fat extraction tube or any other similar apparatus (2) Flasks (3) Weighing balance		
Materials and Reagents	(1) peroxide free Diethyl ether (2) Petroleum ether		
Sample Preparation	**Sugar boiled confectionery & Lozenges** If composition of entire product is desired, grind and mix thoroughly. If product is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as completely as possible, and grind and mix each test portion thoroughly. **Chewing gum and bubble gum** Cut into small bits/ pieces around 50-75 g and mix well. Stored in an airtight container		
Method of analysis	1. Dissolve 10 g sample in 10 mL warm water, and introduce into Mojonnier fat extraction tube or similar apparatus. 2. Add 25 mL peroxide free diethyl ether. 3. Cork the tube and shake vigorously for 1 minute. 4. Add 25 mL of Petroleum ether and shake again for 30 sec. 5. Let stand for 30 min or until separation is complete. 6. Draw off the ether layer containing fat in a previously dried and weighed flask. 7. Repeat the extraction twice. 8. Pool the ether extract, recover excess solvent and dry the fat for 1 h at 100 ^{o}C. Cool and weigh.		

	9. Fat must be dried by keeping the flasks for 30 min and weighed, till constant mass is achieved.
Calculation with units of expression	$$\text{Fat, \% on dry basis} = \frac{M1 \times 100 \times 100}{M2 \times (100 - M)}$$ Where, M1 = Weight in g of the fat M2 = Weight in g of sample taken M = Moisture % in the sample
Inference (Qualitative Analysis)	NA
Reference	IS: 6287- 1985 Methods of Sampling and Analysis for Sugar Confectionery
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Method No.	FSSAI 04C.012:2024	**Revision No. & Date**	0.0
Scope	Sugar boiled confectionery & lozenges		
Caution	Wear mask and gloves during analysis while handling solvents		
Principle	Sample is treated with water, ammonia and ethanol former to dissolve the protein and the latter to help precipitate the proteins and fat is extracted with diethyl ether and petroleum ether. Mixed ethers are evaporated and the residue weighed.		
Apparatus/Instrument	General Apparatus and Glassware (1) Mojonnier fat extraction tube or similar apparatus (2) Flasks. (3) Weighing balance.		
Materials and Reagents	(1) Concentrated Ammonia - sp.gr. 0.88 (2) Ethyl Alcohol 95 to 96 %percent (v/v) (3) Diethyl ether - sp.gr. 0.720 (peroxide free) (4) Petroleum ether – boiling range 40 to 60 ^{o}C, recently distilled		
Sample Preparation	**Sugar boiled confectionery & Lozenges** If composition of entire product is desired, grind and mix thoroughly. If product is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as completely as possible, and grind and mix each test portion thoroughly. **Chewing gum and bubble gum** Cut into small bits/ pieces around 50-75 g and mix well. Stored in an airtight container.		
Method of analysis	1. Introduce 4 g sample into a Mojonnier extraction tube or similar apparatus. 2. Dilute to 10 mL with water. 3. Add 1.2 mL Ammonia solution and mix thoroughly. 4. Add 10 mL alcohol and mix. 5. Then add 25 mL diethyl ether and shake vigorously for about 30 sec and		

	finally add 25 mL petroleum ether and shake again for about 30 sec. 6. Let stand for 20 min or until separation of liquids is complete. 7. Draw off as much as possible of ether fat solution (usually 0.5 to 0.8 mL is left) into a weighed flask through a small rapid filter. 8. Again extract liquid remaining in tube, this time with 15 mL each of ether and petroleum ether; shake vigorously for about 30 sec with each solvent and let settle. Proceed as above, washing mouth of tube and filter with a few mL of mixture of equal parts of two solvents. 9. For accuracy, repeat extraction. If previously solvent-fat solution has been drawn off closely, third extraction usually yields approximately up to 1 mg fat or about 0.02 % with 4 g sample. 10. Slowly evaporate solvent on steam bath and then dry fat in an oven maintained at 100 ^{o}C to constant mass. 11. Test purity of fat by dissolving in a little petroleum ether. If residue remains, wash out fat completely with petroleum ether, dry the residue, weigh and calculate the mass of the fat.
Calculation with units of expression	$$\text{Fat, \% by mass} = \frac{M1}{M2} \times 100$$ Where M1 = Weight in g of the fat M2 = Weight in g of sample taken
Inference (Qualitative Analysis)	NA
Reference	IS: 6287-1985 Methods of Sampling and Analysis for Sugar Confectionery
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Method No.	FSSAI 04C.013:2024	**Revision No. & Date**	0.0
Scope	Chocolate		
Caution	Concentrated Hydrochloric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Chocolates samples are hydrolysed using aqueous acid media. Separated fat is filtered using a filter aid. Fat is extracted using a solvent from filter aid. After solvent evaporation residue is weighed.		
Apparatus/Instrument	General Apparatus and Glassware 1. Buchner funnel – 9 cm size. 2. Soxhelet Apparatus - with 250 mL flat bottom extraction flask. 3. Filter aid - a suitable brand 4. Weighing Balance. 5. Beaker. 6. Steam bath.		
Materials and Reagents	1. Hydrochloric acid - sp.gr. 1.16 2. Petroleum ether - redistilled below 60 °C. 3. Sodium sulphate - anhydrous. 4. Distilled water.		
Sample Preparation	**Chocolate** 1. Melt the product in a beaker at a temperature of 45-50 °C. Pour the melted sample on a marble slab and mix thoroughly with a spatula till the product is solidified and transfer to a stoppered glass bottle. Store in a cool place. 2. Chill the material until hard and then grate or shear to a fine granular condition. Mix thoroughly and transfer to a stoppered glass bottle. Store in a cool place. 3. Alternatively melt in a suitable container by placing container in water bath at about 50 °C. Stir frequently until test portion melts and reaches temperature of 45 – 50 °C, remove from bath, stir thoroughly and while still hot remove test portion for analysis using glass or metallic tube provided with close fitting plunger to expel test portion from tube or disposable plastic syringe.		
Method of analysis	1. Weigh accurately about 10 to 20 g of the prepared sample into a 400mL beaker and add 30 mL of water and 25 mL of hydrochloric acid.		

	2. Heat for 30 min on a steam bath, with frequent stirring. 3. Add 5 g of filter aid and 50 mL of ice-cold water and chill for 30 min in ice-cold water. 4. Fit a heavy piece of linen into the Buchner funnel and moisten with water. 5. Apply gently suction and pour over it a suspension of 3 g of filter aid in 30 mL of water. Filter the hydrolyzed mixture by gentle suction, rinsing the beaker three times with ice-cold water, taking care to leave a layer of liquid on the filter. 6. Finally wash three times with ice-cold water and suck dry. 7. Transfer the filter-cake from the funnel to the original beaker, using a small piece of filter paper to transfer any material adhering to the funnel. 8. Wash the funnel with petroleum ether into the beaker and evaporate the ether on a steam bath. 9. Break up the cake with a glass rod and allow it to remain on the steam bath until the contents are so dry as to enable pulverizing easily. Place in an oven at 100 + 2 °C for one hour. 10. Add 15 g of powdered anhydrous sodium sulphate and mix well. 11. Transfer the mixture to the fat extraction thimble of the Soxhelet apparatus. Wash the beaker with 50 mL of petroleum ether and transfer the washings to the thimble. 12. Extract the fat with petroleum ether so that at least 300 mL has been circulated. 13. Transfer the extract to a tared dish and evaporate the petroleum ether on a steam bath. 14. Dry the fat till the difference in weight between successive weighing is not more than 1 mg. Note: - In case of plain covering chocolate, fat can be extracted directly in a Soxhelet apparatus
Calculation with units of expression	Total Fat % by mass (on moisture free basis) $= \frac{10000 \times w}{W \times (100-M)}$ Where, w = weight in g of fat W = weight in g of prepared sample taken for the test. M = moisture, percent by weight, in the prepared sample.
Inference (Qualitative Analysis)	NA
Reference	IS : 1163 - 2023 Specification for Chocolate
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Method No.	FSSAI 04C.014:2024	**Revision No. & Date**	0.0
Scope	Chocolate		
Caution	Concentrated Conc. Sulphuric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Determination of milk fat is based on extracting total fat followed by estimation of RM value of extracted fat.		
Apparatus/Instrument	General Apparatus and Glassware 1. Soxhlet extraction unit. 2. Flat-Bottom Boiling Flask— The flask (A) shall be made of resistance glass. 3. Still-Head — The still-head (B) shall be made of glass tubing of wall thickness 1.25 ± 0.25 mm. A rubber stopper, fitted below the bulb of the longer arm of the still-head, and used for connecting it to the flask, shall have its lower surface 10 mm above the center of the side-hole of the still-head. 4. Condenser — The condenser (C) shall be made of glass. 5. Receiver — The receiver (D) shall be a flask, with two graduation marks on the neck. 6. Asbestos Board — An asbestos board (E), 120 mm diameter, 6 mm in thickness, with a circular hole about 65 mm in diameter, shall be used to support the flask over the burner. 7. Bunsen Burner 8. Reichert- Meissl Distillation Apparatus 9. Burette. 10. Wire gauge. 11. Watch glass. 12. Graduated flask. 13. Measuring Cylinder. 14. Whatman No. 4 filter paper 15. Pipette		
Materials and Reagents	(1) Glycerin (2) Sodium hydroxide. (3) Pumice Stone Grains —1.4 to 2.0 mm in diameter. (4) Conc. Sulphuric acid. (5) Phenolphthalein Indicator (6) Rectified spirit. (7) Ethyl Alcohol — 90%, v/v neutral to phenolphthalein.		
Preparation of Reagents	1. Conc. NaOH Solution 50 % (w/w): Dissolve NaOH in an equal weight of		

	water and store the solution in a bottle protected from carbon dioxide. Use the clear portion free from deposit. 2. Dilute H_2SO_4 Solution -1 N. 3. Standard NaOH solution-0.1 N. 4. Phenolphthalein Indicator — Dissolve 0.1 g of phenolphthalein in 100mL of 60 % rectified spirit.
Sample Preparation	**Chocolate** 1. Melt the product in a beaker at a temperature of 45-50 °C. Pour the melted sample on a marble slab and mix thoroughly with a spatula till the product is solidified and transfer to a stoppered glass bottle. Store in a cool place. 2. Chill the material until hard and then grate or shear to a fine granular condition. Mix thoroughly and transfer to a stoppered glass bottle. Store in a cool place. 3. Alternatively melt in a suitable container by placing container in water bath at about 50 °C. Stir frequently until test portion melts and reaches temperature of 45 – 50 °C, remove from bath, stir thoroughly and while still hot remove test portion for analysis using glass or metallic tube provided with close fitting plunger to expel test portion from tube or disposable plastic syringe.
Method of analysis	1. Extract sufficient quantity of fat (generally 7 g) from 25-30 g of sample using Soxhlet extraction method. 2. Weigh 5 g of fat and determine R.M Value. 3. Extrapolate the milk fat content from the observed R.M value taking the standard value of 28 for pure milk fat. **Method for Determination of RM Value of extracted fat** 4. Weigh accurately 5.00 ± 0.01 g of the filtered oil or fat into the boiling flask. 5. Add 20 g of glycerol and 2 mL of conc. NaOH solution from a burette to which access of carbon dioxide is prevented and whose orifice is wetted before running in the liquid, the first few drops from the burette being rejected. 6. Heat the flask and its contents with continuous shaking on a gauge over the

	naked flame until the fat, including any drops adhering to the upper parts of the flask, has been saponified and the liquid becomes perfectly clear. Avoid overheating during this saponification. 7. Cover the flask with a watch glass, and allow the flask to cool a little. Add 90 mL of boiling distilled water, which has been vigorously boiled for about 15 min. After thorough mixing, the solution should remain clear. If the solution is not clear (indicating incomplete saponification) or is darker than light yellow (indicating overheating), repeat the saponification with a fresh sample of the oil or fat. If the sample is old, the solution may sometimes be dark and not clear. 8. Add 0.6 to 0.7 g of pumice stone grains and 50 mL of 1N sulphuric acid and immediately connect the flask with the distilling apparatus. Place the flask on the asbestos board. 9. After the fatty acids have melted and separated into a clear liquid layer on gentle warming, heat the flask without altering the flame so that 110 mL of liquid distils over in the course of 19-21 min. 10. The distillation is considered to begin when the first drop forms in the still head. 11. Keep the water flowing in the condenser at a sufficient speed to maintain the temperature of the outgoing water from the condenser between 15 °C and 20 °C. 12. Collect the distillate in a graduated flask. 13. As soon as 110 mL have distilled over, stop heating the boiling flask and replace the graduated flask by a measuring cylinder of about 25 mL capacity to catch washings. 14. Close the graduated flask with the stopper, and, without mixing the contents, place it in a water-bath at 15 °C for 10 min, making sure that the 100 mL graduation mark is below the level of the water. Swirl round the contents of the flask from time to time. 15. Dry the outside of the flask and then mix the distillate by closing the flask and inverting it four or five times, but do not shake. 16. Filter through a dry Whatman No. 4 filter paper or equivalent. Reject the first 2-3 mL of the filtrate and collect the rest in a dry flask. 17. Pipette 100 mL of the filtrate in a titration flask, add 0.1 mL of

	phenolphthalein indicator solution and titrate with standard 0.1 N NaOH solution until the liquid becomes slightly pink. 18. Run a blank test without the fat but using the same quantities of reagents and following the same procedure.
Calculation with units of expression	Milk Fat, % by mass = $\frac{(RV - 0.2) \times F}{26}$ (on dry Basis) Where, RV = Reichert value obtained for extracted fat F = Total Fat % in the sample 0.2 = Reichert value of cocoa butter 26 = Reichert value of milk fat
Inference (Qualitative Analysis)	NA
Reference	IS : 1163- 2023 Specification for chocolates
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.015:2024	**Revision No. & Date**	0.0
Scope	Chocolate		
Caution	Wear mask and gloves during analysis		
Principle	Solvent ether soluble and water soluble components are removed from the chocolates. Remaining residue is calculated as cocoa solids.		
Apparatus/Instrument	General Apparatus and Glassware (Page 3 and 4) 1. Centrifuge with tubes 2. Flat end glass rod. 3. Aluminium dish. 4. Weighing balance. 5. Steam bath. 6. Hot air oven. 7. Desiccator.		
Materials and Reagents	1. Solvent Ether. 2. Sodium oxalate. 3. Distilled water. 4. Alcohol 5. Acetone. 6. Desiccants for Desiccator.		
Preparation of Reagents	1. Sodium oxalate solution (1%, w/v): Sodium oxalate (1g) is dissolved in distilled water (100 mL)		
Sample Preparation	**Chocolate** 1. Melt the product in a beaker at a temperature of 45-50 °C. Pour the melted sample on a marble slab and mix thoroughly with a spatula till the product is solidified and transfer to a stoppered glass bottle. Store in a cool place. 2. Chill the material until hard and then grate or shear to a fine granular condition. Mix thoroughly and transfer to a stoppered glass bottle. Store in a cool place. 3. Alternatively melt in a suitable container by placing container in water bath at about 50 °C. Stir frequently until test portion melts and reaches temperature of 45 – 50 °C, remove from bath, stir thoroughly and while still hot remove test portion for analysis using glass or metallic tube provided with close fitting plunger to expel test portion from tube or		

	disposable plastic syringe.
Method of analysis	1. Treat 50 g milk chocolate with three 100 mL portions of solvent ether in centrifuge bottle, centrifuging and decanting after each addition. 2. Dry residue in bottle and crush to powder with flat end glass rod. Shake with 100 mL 1%, w/v Sodium oxalate and let stand for 30 min. 3. Centrifuge and decant, wash in bottle with three 100 mL portions of water at room temperature shaking well each time until no cocoa material adheres to the bottle. Centrifuge 10 – 15 min after each washing and decant. 4. Wash residue in the same fashion with two 100 mL portions of alcohol and one 100 mL portion of ether. With the aid of small portions of ether, transfer residue resulting from ether, alcohol and aqueous extract to tared aluminium dish provided with tight fit cover. Use small amount of acetone and policeman to transfer any material that sticks to bottom. 5. Evaporate liquid carefully on steam bath and dry residue in oven at 100 °C. 6. Cool dish in desiccator and weigh till constant mass is achieved.
Calculation with units of expression	To obtain moisture free and fat free cocoa mass multiply the weight of residue with factor 1.43. To obtain weight of chocolate liquor multiply the weight of residue with factor 2.2 (This factor is based on fat content of 54% in chocolate liquor).The correction factor is calculate by using said formula.
Inference (Qualitative Analysis)	NA
Reference	A.O.A.C 21st edn, Official Method of Analysis(2019) Method no.931.05 Cocoa solids of chocolate liquor
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.016:2024	**Revision No. & Date**	0.0
Scope	Chocolate		
Caution	Concentrated sulphuric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Initially fat is removed by extraction. Non-fat milk solids are precipitated and estimated.		
Apparatus/Instrument	General Apparatus and Glassware 1. Weighing balance 2. Pipette 3. Buchner funnel – 7 cm 4. Kjeldahl flask 5. Round Bottom Flask 6. Pumice stones 7. Kjeldahl distillation system		
Materials and Reagents	1. Petroleum ether 2. Sodium Oxalate 3. Glacial acetic acid 4. Tannic acid 5. Concentrated sulphuric acid- sp. Gr. 1.84 6. Selenium 7. Mercuric oxide 8. Sodium hydroxide 9. Sodium thiosulphate 10. Methyl Red Indicator 11. Rectified spirit (95 percent by volume). 12. Sodium sulphate.		
Preparation of Reagents	1. Sodium Oxalate solution – Approximately 1% percent (w/v) 2. Tannic acid solution- approximately 10 percent (w/v). 3. Catalyst mixture- 1.0 g of Selenium and 5.0 g of Mercuric oxide intimately mixed together. 4. Alkali solution- prepared by dissolving 300 g of sodium hydroxide and		

	10 g of sodium thiosulphate in 500 mL of water. 5. Standard Sulphuric acid-Approximately 0.1 N. 6. Methyl Red Indicator solution- Dissolve one gram of methyl red in 200 mL of rectified spirit (95 percent by volume). 7. Standard Sodium hydroxide Solution- approximately 0.1 N.
Sample Preparation	**Chocolate** 1. Melt the product in a beaker at a temperature of 45-50 °C. Pour the melted sample on a marble slab and mix thoroughly with a spatula till the product is solidified and transfer to a stoppered glass bottle. Store in a cool place. 2. Chill the material until hard and then grate or shear to a fine granular condition. Mix thoroughly and transfer to a stoppered glass bottle. Store in a cool place. 3. Alternatively melt in a suitable container by placing container in water bath at about 50 °C. Stir frequently until test portion melts and reaches temperature of 45 – 50 °C, remove from bath, stir thoroughly and while still hot remove test portion for analysis using glass or metallic tube provided with close fitting plunger to expel test portion from tube or disposable plastic syringe.
Method of analysis	1. Weigh accurately about 10 g of the prepared sample and extract the fat by shaking and centrifuging with two consecutive portions each of 100 mL of petroleum ether. Remove the last traces of ether from the extracted residue in an air oven. 2. Shake the de-fatted residue with 100 mL of water for 4 min and then add 100 mL of sodium oxalate solution. Stopper and shake vigorously for 3 min. 3. Allow this mixture to stand for 10 min, shake again for 2 min and then centrifuge for 15 min. 4. Pipette 100 mL of the clear supernatant liquid into 250 mL beaker and add 1 mL of glacial acetic acid, stir gently, stand for a few min 5. Add 4 mL of freshly prepared Tannic acid solution and stir. 6. Allow the precipitate to settle and filter through a Whatman filter paper No. 42 or equivalent overlaid with paper pulp, in a 7 cm Buchner funnel, wash twice with the sodium oxalate solution containing 1% (w/v) of the glacial

	acetic acid and 2% w/v of tannic acid solution. 7. Digest the precipitate in a Kjeldahl flask with 20 mL of sulphuric acid, 15 g of sodium sulphate and 1 g of the catalyst, for 30 min after the mixture has become clear. 8. Cool the contents of the flask. Transfer quantitatively to a round-bottom flask, with water, the total quantity used being about 200 mL. Add with shaking a few pieces of pumice stone to prevent bumping. 9. Add 50 mL of alkali solution carefully over the side of the flask so that it does not mix at once with the acid solution but forms a layer below the acid. 10. Assemble the apparatus, taking care that the tip of the condenser extends below the surface of the sulfuric acid contained in the beaker. 11. Mix the contents of the flask by shaking and distill until all ammonia has distilled over into the standard sulfuric acid. 12. Detach the flask from the condenser and shut off the burner. Rinse the condenser thoroughly with water into the beaker. Wash the tip carefully so that all traces of condensate are transferred to the beaker. 13. When all the washings have drained into the beaker, add 2-3 drops of the methyl red indicator solution and titrate with standard sodium hydroxide solution. 14. Carry out a blank using all reagents in the same quantities but without the sample to be tested.
Calculation with units of expression	Non- fat milk solids % by mass = $\frac{3126.2 \times (B-A) \times N}{M}$ Where, B = volume in mL of standard sodium hydroxide solution used to neutralize the acid in the blank determination; A = volume in mL of standard sodium hydroxide solution used to neutralize the excess of acid in the test with the material; N = normality of standard sodium hydroxide solution; and M = mass in g of the material taken for the test
Inference (Qualitative Analysis)	NA
Reference	I.S 1163: 2023 Specification for Chocolate
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Method No.	FSSAI 04C.017:2024	**Revision No. & Date**	0.0
Scope	Cocoa Powder, Dry mixtures of cocoa and sugars, chocolate powder		
Caution	Wear mask and gloves during analysis		
Principle	Cocoa butter is extracted using solvent extraction technique.		
Materials and Reagents	1. Petroleum ether		
Preparation of Reagents	1. Petroleum ether – Petroleum ether is distilled. Distillate is collected below 60 °C.		
Sample Preparation	Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, after withdrawl of test portions for analytical determinations.		
Method of analysis	1. Weigh accurately 5 to 10 g of the moisture free material 2. . transfer it to the fat extraction thimble of the Soxhlet apparatus 3. Extract the fat with petroleum ether for about 16 hours 4. Continue the extraction till at least 300 ml of petroleum ether have been circulated. 5. Dry the fat (cocoa butter) at 70°C in a vacuum oven or at 100-110°C in an air oven till the difference in mass between two successive weighings is not more than 1 mg.		
Calculation with units of expression	Cocoa butter (%) on moisture free basis = $\frac{\text{Wt of extracted fat x100 x 100}}{\text{Wt of sample (100 – Moisture)}}$		
Inference (Qualitative Analysis)	NA		
Reference	IS : 1164-1986 Specification for Cocoa Powder		
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Method No.	FSSAI 04C.018:2024	Revision No. & Date	0.0
Scope	Cocoa mass or cocoa/chocolate Liquor and cocoa cake		
Caution	Concentrated Hydrochloric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	The fat in cocoa mass (cocoa/chocolate liquor) is enclosed by cellulose. It is liberated by digesting with hydrochloric acid. The digested material is filtered in a suitable manner to remove the acid solution and the fat is then extracted in a soxhlet apparatus. residual fat in the flask is dried and calculated as cocoa butter content.		
Apparatus/Instrument	General Apparatus and Glassware 1. Soxhlet apparatus – with 250 mL flat bottomed flask. 2. Thimble. 3. Oven. 4. Solvent distillation system. 5. Steam bath.		
Materials and Reagents	1. Petroleum ether, dried, freshly distilled, boiling point below 60 °C. 2. Hydrochloric acid, chemically pure, 25 percent by weight (Sp Gr 1.12) 3. 0.1 N silver nitrate solution (AgNO3)		
Preparation of Reagents	1 Petroleum ether – Petroleum ether is distilled. Distillate is collected below 60 °C. 2 Hydrochloric acid, chemically pure, 25 percent by weight (Sp Gr 1.12) 3 0.1 N silver nitrate solution (AgNO3)		
Sample Preparation	Cool the material until hard and then grate or shear to a fine granular condition.		
Method of analysis	**1) Digesting the Sample** a. Weigh 3 to 4 g of sample in a 500 ml beaker accurately.. b. Add 45 ml of boiling hot distilled water into the beaker and stir to give a homogenous suspension. c. Again stirring continuously, add 55 ml of 25 percent hydrochloric acid (giving 4 N HCl).		

	d. Add a few defatted, incinerated pieces of pumice stone or pumice powder. e. Cover the beaker with a watch glass or connect it to a reflux condenser in order to avoid losses by splashing and to prevent the acid from becoming too concentrated due to evaporation of the water. f. Bring the contents of the beaker slowly to boiling point. When boiling starts, remove the flame momentarily to avoid overflowing. g. Boil the contents gently for about 15 min. Rinse watch glass or condenser used above into the beaker with 100 ml of boiling water. h. Filter the digest while still hot through a wetted, fat free, fluted filter paper of such a pore size as to allow the filtration to proceed at a reasonable speed. i. Wash the beaker several times with hot water and also pass the washings through the filter paper. j. Wash the filter paper with several further lots of hot water until the filtrate ceases to give a chloride reaction with silver nitrate. k. While still wet, transfer the filter paper with sample to a defatted extraction thimble and dry in a small beaker for up to 6 h at 100-101 °C. **1) Extraction** a. Place a few pieces of pumice stone into a 250 ml flat bottomed Soxhlet flask and dry for 1 h in an oven at 100-101 °C. b. Cool the flask in a desiccators for 30 min and accurately weigh on an analytical balance. c. Place the thimble containing the dried filter paper with the digested sample into a soxhlet extractor. d. Rinse the beaker (dried) which was used for the digestion several times with petroleum ether and pour the washings into the thistle with soxhlet extractor. e. Extract the digested sample and the filter paper under a reflux condenser for 4 h using 40-50 ml petroleum ether. f. After completion, distil off the petroleum ether on a water bath and dry the flask with the fat, lying, on its side, either under vacuum at 70 °C, or in on an oven at 100-101 °C. g. After drying, remove the last traces of ether by blowing air into the flask using a rubber balloon.

	h. Cool the flask for 30 minutes in a desiccator at room temperature. i. Reweigh on an analytical balance.
Calculation with units of expression	$\text{Cocoa butter (\%) on moisture free basis} = \frac{A \times 100 \times 100}{M_1 * S}$ Where A = extracted fat in the flask, in g; M_1 = mass of sample in g; and S = percent dry matter, in sample.
Inference (Qualitative Analysis)	NA
Reference	IS-11923|:2022 Cocoa Mass (Cocoa/Chocolate Liquor) — Specification
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Method No.	FSSAI 04C.019:2024	**Revision No. & Date**	0.0
Scope	Cocoa Powder, Cocoa mass or cocoa/chocolate Liquor and cocoa cake		
Caution	Concentrated sulphuric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Crude fiber (CF) is the residue of plant food left after extraction by dilute acid followed by dilute alkali and residue is heated in a muffle furnace. Loss in residue is calculated as crude fibre.		
Apparatus/Instrument	General Apparatus and Glassware. 1. Condenser – Use condenser that will maintain constant volume of refluxing solutions. 2. Digestion flask/Beaker – 1000 mL Erlenmeyer flask/Long Beaker is recommended. 3. Filtering cloth –Use filtering cloth of such character that no solid matter passes through when filtering is rapid. Fine linen or dress linen with about18 threads/cm or 45 threads per inch (i.e. the aperture size 0.14 mm and thread thickness 0.42 mm) or its equivalent may be used (Whatman filter Paper No. 54 or equivalent may also be used). 4. Muffle Furnace maintained at 525 ± 20 °C.		
Materials and Reagents	1. Sulphuric acid. 2. Caustic soda (free from sodium carbonate). 3. Ether-(Solvent grade). 4. Ethyl Alcohol 5. Sodium Oxalate Solution-one percent(m/v) 1%		
Preparation of Reagents	1. Sulphuric acid (1.25%) - Sulphuric acid (1.25 g) dissolved in distilled water (100 mL) (w / v). 2. Caustic soda (1.25%) - Caustic soda (1.25 g) dissolved in distilled water (100 mL) (w / v). 3. Sodium Oxalate Solution— one percent (m/v). Dissolve 1.0 (g) in distilled water(100 mL)		

Sample Preparation	Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, after withdrawal of test portions for analytical determinations.
Method of analysis	**(A) Preparation of Moisture and Fat Free Sample-** 1. Weigh 7 g of prepared sample in centrifuge tube and treat twice with 100 mL of ether. 2. Centrifuge the tube and decant supernatant liquid(supernatant means upper liquid layer) after each addition of ether. 3. Dry the residue (pellet) in an air-oven at 100 ± 2 °C and powder it with a flattened glass rod. 4. Add 100 mL of water, centrifuge for 10 min and decant the aqueous layer. 5. Repeat the washing with water twice. 6. Then wash twice with 100 mL of alcohol and once with 100 mL ether, in the same manner as done with water. 7. Transfer the residue to a dish and dry in an air-oven at 105 ± 1 °C till the difference in mass between two successive weighing is not more than 1 mg. **(B) Procedure** 1. Weigh accurately about 2.5 g of the moisture and fat-free material and transfer to a L flask/beaker 2. Take 200 mL of dilute sulphuric acid in a beaker and bring to boil. 3. Transfer all the boiling sulphuric acid to the flask containing the fat-free material and immediately connect with reflux water condenser. 4. Heat, so that the contents of the flask begin to boil within 1 min. 5. Rotate the flask frequently, taking care to keep the material from remaining on the sides of the flask out of contact with the acid. 6. Continue boiling exactly for 30 min. 7. Remove the flask and filter through fine linen (about 18 threads to a centimetre) held on a funnel. 8. Wash with boiling water until the washings are no longer acidic to litmus.

	9. Heat some quantity of sodium hydroxide solution to boiling under a reflux condense. 10. Wash the residue on the linen into the L flask with 200 mL of the boiling sodium hydroxide solution. 11. Immediately connect the flask with the reflux condenser and boil for exactly 30 min. 12. Remove the flask and immediately filter through the filtering cloth (about 18 threads to a centimetre) held on a funnel. 13. Thoroughly wash the residue with boiling water and transfer to a Gooch crucible prepared with a thin but compact layer of the ignited asbestos. 14. Wash the residue thoroughly first with hot water and then with about 15 mL of ethyl alcohol (95% by volume). 15. Dry the Gooch crucible with contents at 105 °C in an air-oven to constant mass. Cool and weigh (W_1) 16. Incinerate the contents of the Gooch crucible in an electric muffle furnace at 600 ± 20 °C until all the carbonaceous matter is burnt. 17. Cool the Gooch crucible containing the ash in a desiccator and weigh.(W_2)
Calculation with units of expression	Crude fibre % = (on moisture &fat free base) $\frac{(W_1 - W_2) \times 100}{\text{Wt. of sample}}$
Inference (Qualitative Analysis)	NA
Reference	IS : 1164-1986 Cocoa Powder IS-11923|:2022 Cocoa Mass (Cocoa/Chocolate Liquor) — Specification
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.020:2024	**Revision No. & Date**	0.0
Scope	Chocolate		
Caution	Wear gloves during analysis		
Principle	Determination of Non-cocoa butter is based on solvent extraction of the fat, separation of the sterene fraction, and analysis of individual sterens with mass spectrometric detection.		
Apparatus/Instrument	General Apparatus and Glassware 1. Beaker 2. Lipid fractionation chromatographic column (1.5cm id by 50cm) fitted with polytetrafluoroethylene stopcock. 3. Gas Chromatograph a). Carlo Erba 4160 gas chromatograph or similar equipment capable of reproducing the following conditions: oven temperatureof 200 to 280˚C at a rate of 7˚C/min. b) Column: 50m x 0.32mm column with a 0.2 µm polar stationary phase (e.g J& W DBWax, CP Wax, 52 CB) c) VG 12-250 Quadrupole mass spectrometer or comparable model 4. Spatula, warmed 5. Soxhlet thimble 6. Cellulose filter paper		
Materials and Reagents	1. Silica gel 60, 7.-230mesh 2. Anhydrous Sodium Sulfate 3. Hexane 4. Stigmasta-3,5,22-triene (92%) 5. Cholesta-3,5-diene 6. Campesta-3,5-diene plus Stigmasta-3,5-diene (97%) 7. Isooctane		

	8. Celite 9. Dichloromethane
Sample Preparation	Chocolate sample should be chilled in a refrigerator, grated to a powder and mixed.
Method of analysis	1. Place a 2.5g test portion in a beaker 2. Add internal standard (cholestadiene ,5µg, in 0.05ml isooctane 3. Melt chocolate at 55˚C for 5 minutes. 4. Add Celite, 3g at 55˚C and mix with a warm spatula. 5. Place mixture in a Soxhlet thimble and extract overnight with 150ml dichloromethane 6. Filter extract through a cellulose filter paper and evaporate solvent 7. Transfer residue to a fractionating column 8. Column Chromatography: a. Top Column with 0.5cm anhydrous sodium sulfate. Transfer a 1g sample to the fractionating column with three 1ml volume of hexane. b. Elute column with hexane at a flow rate of approximately 2ml/min c. Discard the first 25mL fraction (alkanes). d. Collect the next 40mL fraction (sterenes). e. Evaporate the hexane and transfer the residue to a 1mL vial with three 0.3mL volumes of hexane. f. Evaporate the solution to dryness and re-dissolve in 0.1mL isooctane prior to GCMS.
Calculation with units of expression	1. Calibration curve preparation a. Curve should be prepared such that they are linear upto 100µg/mL sterene with correlation coefficient ® of typically 0.996 2. GCMS Spectra a. Scan spectra in electron ionization mode at 200˚C with an electron current of 70ev and a trap current of 200 microamps. Acquire spectra over the mass range of 35 to 600 daltons at a rate of 0.7s/scan with an interscan delay of 0.5ms.
Inference (Quantitative Analysis)	a) Analysis is done by selected ion monitoring. Molecular ions of cholestadiene (m/z 368), Campestadiene (m/z 382), Stigmastatriene (m/z

	394), and Stigmastadiene (m/z 396) were selected. Ions corresponding to (M-alkyl)+ and the common ring fragment ions at m/z 255 and m/z 275 should be monitored to confirm peak identity. Campestatriene (m/z 380) and ions thought to be derived from non-polar degradation products of triterpene alcohols (m/z 2=189,218,365,393, and 408) may also be monitored. b) Calibration graphs should be constructed and their slope as used to calculate the sterene content of the test portion. Calibration should be linear up to 100µg/mL sterene with correlation coefficient r, typically 0.996 for each analyte. c) Sterene should be quantified on the basis of peak area measurements relative to the internal standard using the response in the molecular ion channel of each sterene.
Reference	AOCS Ce 10/02 - Non-Cocoa Butter Vegetable Fats and Oils in Chocolate by the Analysis of Hydrocarbon Sterol Degradation Products
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.021:2024	**Revision No. & Date**	0.0
Scope	Sugar Boiled Confectionery & Lozenges		
Caution	Concentrated sulphuric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Initially, Starch is separated from other ingredients (viz., sugars, fat etc) and hydrolysis is done and Resultant glucose is estimated and starch content is calculated using the factor.		
Apparatus/Instrument	General Apparatus and Glassware 1. Electric mixer 2. Filtration set 3. Test tubes.		
Materials and Reagents	1. Alcohol 2. Distilled water. 3. α-naphthol 4. Conc. Sulphuric acid		
Preparation of Reagents	Malt extract – Prepare infusion of freshly ground malt just before use. For every 80 mL malt extract required digest 5 g ground malt with 100 mL water at room temperature for 2 h (or 20 min if mixture can be stirred by electric mixer). Filter to obtain clear extract, re-filtering first portion of filtrate if necessary. Mix infusion well.		
Sample Preparation	**Sugar boiled confectionery & Lozenges** If composition of entire product is desired, grind and mix thoroughly. If product is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as completely as possible, and grind and mix each test portion thoroughly.		
Method of analysis	1. Measure 25 mL of solution of uniform mixture (representing 5 g test portion) into 30 mL beaker, or add to beaker 5 g finely ground test portion (previously extracted with ether, if test sample contains much fat); add enough water to make 100 mL; heat to 60 °C (avoiding, if possible		

	gelatinizing starch); and let stand for 1 h, stirring frequently to secure complete solution of sugar. 2. Transfer to wide-mouth bottle, rinse beaker with little warm water and cool. 3. Add equal volume of alcohol and mix and let stand for about an hour. 4. Centrifuge until precipitate is closely packed on bottom of bottle and decant supernatant through hardened filter. 5. Wash precipitate with successive 50 mL portions of alcohol, 50% by volume, by centrifuging and decanting through filter until washings are sugar-free by following test: **Test for sugars** Add to test-tube few drops of washing, 3-4 drops 20% alcoholic α-naphthol solution, and 2 mL water. Shake well, tip tube, let 2 to 5 mL H_2SO_4 flow down sides of tube, and then hold tube upright. If sugar is present, inter phase of two liquids is coloured faint to deep violet; on shaking, whole solution becomes blue-violet. 6. Transfer residue from bottle and hardened filter to beaker with 50 mL water. 7. Immerse beaker in boiling water, and stir constantly 15 min or until, all starch is gelatinized. 8. Cool to 55 °C, add 20 mL malt extract, and hold at this temperature for 1 h, or until residue treated with iodine solution shows no blue tinge upon microscopic examination. 9. Cool, dilute to 250 mL and filter. 10. Place 200 mL of filtrate in flask and add 20 mL HCl (sp. Gr. 1.125), connect with reflux condenser, and heat in boiling water-bath 2.5 h. 11. Cool, nearly neutralize with 10%, w/v NaOH solution, finish neutralization with Na_2CO_3 solution, and dilute to 500 mL. 12. Mix solution thoroughly, pour through dry filter, and determine glucose in aliquots by Munson – Walker method. Conduct Blank determination on same volume of malt extract as used with test portion and correct weighed glucose accordingly.
Calculation with units of expression	Weighed glucose obtained x 0.925 = Weighed Starch
Inference	NA

(Qualitative Analysis)	
Reference	A.O.A.C 21st edn, Official Method of Analysis (2019) Method no.925.50, Starch in Confectionery.
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.022:2024	**Revision No. & Date**	0.0
Scope	Sugar Boiled Confectionery & Lozenges, Chewing Gum And Bubble Gum, Cocoa Powder, cocoa mass/cake		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion **2.** Wear gloves and face protection while doing analysis.		
Principle	Synthetic acidic colour(s) is dyed on to wool in acidic medium and extracted (stripped) from the wool into aqueous alkaline medium. If the wool is not dyed then report absence of added artificial colouring matter. If the wool is dyed, it indicates the presence of a coal-tar dye. Acidic coal tar dyes are permitted and basic coal tar dyes are non permitted colors.		
Apparatus/Instrument	General Apparatus and Glassware (1) Pipette (2) Beaker (3) Flask. (4) Soxlet extractor. (5) Whatmman No.1 filter paper. (6) Wollenthread.		
Materials and Reagents	1. White knitting wool. 2. Petroleum ether. 3. Sodium hydroxide. 4. Distilled water. 5. Ammonia (0.88 sp. gr). 6. Sodium chloride. 7. Acetic acid. 8. Isobutanol. 9. Butanol. 10. Phenol.		
Preparation of Reagents	(1) White knitting wool: - Extract pure white wool in a soxhlet extractor with petroleum ether for 2-3 h to remove fat. Boil in very dilute solution of sodiumhydroxide and then in water to free it from alkali. (2) Paper: Whatman No. 1 chromatographic paper or equivalent. (3) 1 mL (0.88 sp. gr) ammonia + 99 mL water. (4) 2.5% aqueous sodiumchloride . (5) 2% sodiumchloride in 50%, v/v ethanol. (6) Acetic acid solution in water (1:3). (7) Iso-butanol-ethanol-water (1: 2 : 1, v/v). (8) n-butanol-water-glacial acetic acid (20 : 12 : 5, v/v) . (9) Iso-butanol-ethanol-water (3: 2: 2, v/v): to 99 mL of this add 1mL of (0.88 sp		

	gr.) ammonia. (10) 80 g phenol in 20 g water.
Sample Preparation	**Part A** **Cocoa powder** Mix thoroughly and preserve in tightly stoppered bottle. **Sugar boiled confectionery & Lozenges** If composition of entire product is desired, grind and mix thoroughly. If product is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as completely as possible, and grind and mix each test portion thoroughly. **Part B** 1. *Preliminary treatment of food*: Assuming that an acidic colour is present, the preliminary treatment involves removing interfering substances and obtaining the dye in acid solution prior to boiling with wool. 2. Non-alcoholic beverages e.g. soft drinks: As most foods in this group are acidic they can be usually treated directly with wool, otherwise, slightly acidify the food with acetic acid. 3. Alcoholic liquids (e.g. Wine): Boil to remove alcohol and acidify if necessary as in (2). 4. Starch based foods (e.g. cakes, custard powder etc): Grind 10 g of sample thoroughly with 50 mL of 2 % ammonia in 70% alcohol, and allow it to stand for an hour and centrifuge. Pour the separated liquid into a dish and evaporate on water bath. Take up the residue in 30 mL dilute acetic acid. 5. Candied fruits: Treat as in (4). 6. Products with high fat content (e.g. Sausages, meat, fish paste): De-fat the sample with light petroleum and extract the colour with hot water (acidify etc. as usual). Note that oil soluble colours tend to give coloured solutions in organic solvents. 7. If the extraction is difficult treat with warm 50-90% acetone or alcohol (which precipitates starch) containing 2% ammonia. The organic solvent should be removed before acidifying as in (4).
Method of analysis	Extraction of the colour from the food: Acidic Dyes 1. Introduce about 20 cm length of woollen thread into a beaker containing about 35 mL of the prepared acidified solution of the sample and boil for a few min till the woollen thread is dyed. 2. Take out the woollen thread and wash it with tap water.

	3. Transfer the washed woollen thread to a small beaker containing dilute ammonia and heat again. If the colour is stripped by the alkali, the presence of an acid coal-tar dye is indicated. 4. Remove the woollen thread. Make the liquid slightly acidic and boil with a fresh piece of woollen thread. Continue boiling until the colour is taken by the woollen thread. 5. Extract the dye from the woollen thread again with a small volume of dilute ammonia, filter through a small plug of cotton and concentrate the filtrate over a hot water bath. 6. This double stripping technique usually gives a pure colour extract. Natural colours may also dye the wool during the first treatment, but the colour is not usually removed by ammonia. Basic dyes 7. Basic dyes can be extracted by making the food alkaline with ammonia, boiling with wool and then stripping with dilute acetic-acid. 8. At present, all the permitted water soluble coal-tar dyes are acidic, hence an indication of the presence of a basic dye suggests that an unpermitted colour is present.
Inference (Qualitative Analysis)	If the wool is dyed, it indicates the presence of a coal-tar dye. Presence of basic coal tar dyes indicate the presence of non permitted colors.
Reference	Manual Methods of Analysis for Adulterants and Contaminants in Food, I.C.M.R 1990
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.023:2024	**Revision No. & Date**	0.0
Scope	Sugar Boiled Confectionery & Lozenges, Chewing Gum And Bubble Gum, Chocolate, Cocoa powder		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Wear gloves and face protection during handling of acid and solvents.		
Principle	Filth analysis is a method used to detect and count light solid impurities of mineral, vegetable, or animal origin in food products. Types of filth may include dirt, soil particle, insects fragments, rodent hair & excreta etc.		
Apparatus/Instrument	General Apparatus and Glassware (Page 3 and 4) (1) Microscope (2) Magnetic stirrer – hot plates (3) Sieves set. (4) Wire basket – 8 cm diameter and 3 cm height, made from No. 8 screen and with wire handles. (5) Hirsch funnel. (6) Water bath with heating system / steam bath. (7) Trap flasks		
Materials and Reagents	(1) Hydrochloric acid (2) Turgitol anionic 7 –sodium heptadecyl sulphate (3) Mineral oil – Paraffin oil, white, light, sp. gravity 0.840-0.860 (24) (4) Isopropanol (5) Chloroform (6) Floatation liquid – mineral oil and heptane (85 +15) (7) Dichloromethane		
Preparation of Reagents	1. Hydrochloric acid is diluted 70 times with distilled water.		
Sample Preparation	**Sugar boiled confectionery & Lozenges** If composition of entire product is desired, grind and mix thoroughly. If product is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as		

	completely as possible, and grind and mix each test portion thoroughly. **Chewing gum and bubble gum** Cut into small bits/ pieces around 50-75 g and mix well. Stored in an airtight container.
Method of analysis	(i) In hard candy, gum drops, gum, starch or pectin based candies 1. Dissolve in boiling HCl. 2. Filter through rapid paper on Hirsch funnel. 3. Examine microscopically. (ii) In hard boiled candy it is difficult to filter. Follow the procedure: 1. Weigh 225 g test portion into 1.5 to 2 L beaker. 2. Add 1 L 5% solution of turgitol and heat in steam bath for 10 min. Stir 5 to 10 min on magnetic stirrer – hot plate. 3. Sieve portion wise on number 230 sieve. If residue on sieve is small, transfer directly to ruled filter paper; otherwise, transfer quantitatively to 2 L trap flask, using 40%, v/v isopropanol. 4. Bring volume to 1 L with 40%, v/v isopropanol and add 50 mL HCl. 5. Gently stir on magnetic stirrer – hot plate, while heating to full boil. 6. Immediately transfer flask to cool stirring unit and add 40 mL light mineral oil. 7. Stir magnetically for 2 min, let it stand for 1 minute; then slowly fill flask with 40%, v/v isopropanol by running liquid down stoppered rod while top of stopper is maintained just above liquid. After filling flask, gently stir settled plant material for 5 to 10 sec with stoppered rod. 8. Let stand undisturbed for 2 min and immediately trap off. 9. Add 25 mL light mineral oil, stir by hand gently for 30 sec, and let stand for 10 min. Repeat trapping. Wash flask neck thoroughly with isopropanol and transfer washings to beaker containing trappings. 10. Filter onto ruled paper and examine microscopically. (iii) In water insoluble candies containing confectioner's corn flakes, wheat bran, or other cereal fillers, and those whose major constituents, excluding the chocolate coating, consists primarily of finely ground nutmeats (e.g., Peanut butter, almond paste etc.) excluding the chocolate coating . Follow the

	procedure: 1. Proceed as mentioned in (ii); through sieving on number 230 sieve. 2. Wash residue on sieve with isopropanol. 3. Form filter paper around 600 mL beaker, moistening with water to make paper pliable. Insert paper into 91 mm buchner, wash with isopropanol, and aspirate to near dryness. 4. Quantitatively transfer residue on sieve to filter paper cup with isopropanol and add enough isopropanol to cover residue. 5. After 1 minute apply vacuum until dripping ceases. 6. Place paper cup containing sieved residue in 1 L beaker, add 200 mL chloroform, and boil 5 min on steam bath. 7. After few min of cooling, lift paper, drain, and transfer to 200 mL fresh chloroform. Repeat for 5 min, boil and drain. 8. Return paper cup to buchner and apply vacuum until dripping ceases. Cover residue with isopropanol for 5 min, reapply vacuum, and continue to aspirate for 5 min after visible dripping ceases. 9. Transfer quantitatively to 2 L trap flask, using 40%, v/v isopropanol. Bring volume to 1 L with 40%, v/v isopropanol and add 50 mL HCl. 10. Gently stir on magnetic stirrer – hot plate, while heating to full boil. 11. Immediately transfer flask to cool stirring unit and add 40 mL floatation liquid. 12. Stir magnetically for 2 min, let it stand for 1 minute; then slowly fill flask with 40%, v/v isopropanol by running liquid down stoppered rod while top of stopper is maintained just above liquid. After filling flask, gently stir settled plant material for 5 to 10 sec with stoppered rod. 13. Let stand undisturbed for 2 min and immediately trap off. 14. Add 25 mL floatation liquid, stir by hand gently for 30 sec, and let stand for 10 min. Repeat trapping. Wash flask neck thoroughly with isopropanol and transfer washings to beaker containing trappings. 15. Filter onto ruled paper and examine microscopically. (iv) In chocolate candy coating 1. Heat 400 mL dichloromethane in 800 mL beaker to 30-35 °C and keep at this temperature.

	2. Place test portion of candy in wire basket. 3. Move basket up and down through dichloromethane until chocolate coating dissolves. Rinse each candy center with fine stream of dichloromethane from wash bottle and save center. Repeat with balance of test sample. 4. Stir dichloromethane-chocolate suspension and pour through No. 140 sieve. 5. Transfer residue from sieve to filter paper and examine microscopically. Examine candy centers by appropriate method as in (i), (ii) and (iii).
Inference (Qualitative Analysis)	NA
Reference	A.O.A.C 22nd edn, Official Method of Analysis(2019) Method 971.34 Filth in candy (Floatation method)
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Method No.	FSSAI 04C.024:2024	**Revision No. & Date**	0.0
Scope	Chocolate		
Caution	Rancidity in chocolates is due to degradation of fats present		
Principle	Aldehydes and ketones formed during oxidation of fats react with phloroglucinol developing a pink colour.		
Apparatus/Instrument	General Apparatus and Glassware		
Materials and Reagents	1. Phloroglucin dehydrate 2. Diethyl ether		
Preparation of Reagents	1. Phloroglucin dihydrate: 0.1% in Diethyl ether		
Sample Preparation	Refer 3.0		
Method of analysis	1. Take 10 g of prepared sample. 2. Add 10 mL of 0.1% Phloroglucin dihydrate solution. 3. Appearance of pink colour indicates presence of rancidity.		
Reference	IS 7679-2017 Specification for Hair Creams.		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

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Method No.	FSSAI 04C.025:2024	**Revision No. & Date**	0.0
Scope	Sugar Boiled Confectionery & Lozenges		
Caution	Wear Gloves and Mask while handling solvent or other chemicals		
Principle	Paraffin is extracted with ether and subjected to saponification. Solids from saponified material are extracted with petroleum ether. Material extracted residue is calculated as paraffin.		
Apparatus/Instrument	General Apparatus and Glassware 1. Reflux condenser. 2. Water bath with heating system. 3. Weighing balance.		
Materials and Reagents	1. Alcohol. 2. Sodium hydroxide. 3. Distilled water. 4. Petroleum ether.		
Preparation of Reagents	1. Sodium hydroxide solution: Dissolve sodium hydroxide in equal quantity of water.		
Sample Preparation	**Ether extract of Confectionary** 5. Take 4.0 g test portion, or amount of uniform solution equivalent to this weight dry substance, into Mojonnier fat extraction tube or similar apparatus. 6. Dilute to 10mL with add 1.25 mL NH_4OH, and mix thoroughly. 7. Add 10 mL alcohol and mix; then add 25 mL ether and shake vigorously ca 30s. 8. Add 25 mL petroleum ether (bp<60^oC) and shake again ca 30 s. Let stand 20 min or until serration of liquid is complete 9. Draw off as much as possible of ether-fat solution (usually0.5-0.8mL is left) into flak through small, rapid filter.(Weigh flask with similar one as counterpoise). 10. Again extract liquid remaining in tube, this time with 15mL each of ether		

	and petroleum ether; shake vigorously ca 30 s with each solvent and let settle. 11. Proceed as above, washing mouth of tube and filter with few mL of mixture of equal parts of the 2 solvents (previously mixed and freed from deposited water) 12. For greater degree of accuracy, repeat extraction. 13. If previous solvent-fat solution have been drawn off closely, third extraction usually yields ≤ 1 mg fat, or 0.02% with 4.0 g test portion.
Method of analysis	1. To solvent extract in flask (as obtained in the above method for Ether Extract), add 10 mL alcohol and 2 mL NaOH solution (1+1); connect flask with reflux condenser and heat for 1 h on water-bath or until saponification is complete. 2. Remove condenser and keep flask on bath until alcohol evaporates and residue is dry. 3. Dissolve residue as completely as possible in approximately 40 mL water and heat on bath, shaking frequently. 4. Wash into separator, cool, and extract with 4 successive portions of petroleum ether, collecting extracts in weighed flask (W) or capsule. 5. Evaporate petroleum ether and dry to constant weight (W_1) at 100 °C. Any phytosterol or cholesterol present in fat could be extracted with the paraffin, but the amount is so insignificant that it may generally be disregarded.
Calculation with units of expression	$\% \text{ Paraffin} = \frac{(W_1 - W) \times 100}{M}$ W = weight of the empty flask W_1 = weight of the flask with paraffin M=weight of the sample taken
Inference (Qualitative Analysis)	NA
Reference	A.O.A.C 21stedn, Official Method of Analysis (2019) Method no.920.177 & 920.178 Sugar and sugar products.
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Method No.	FSSAI 04C.026:2024	**Revision No. & Date**	0.0
Scope	Sugar Boiled Confectionery & Lozenges		
Caution	Wear Gloves and Mask while handling solvent or other chemicals		
Principle	Shellac is extracted from confectionery using benzene alcohol mixture. Any fat extracted is removed by petroleum ether wash. Any sugar extracted is removed by water wash.		
Apparatus/Instrument	General Apparatus and Glassware 1. Beaker 2. Weighing balance 3. Watch glass 4. Steam bath 5. Glass dish with flat bottom (7 cm dia.)		
Materials and Reagents	1. Benzene. 2. Absolute alcohol. 3. Petroleum ether. 4. Iso-amyl alcohol (B.P. – 129-132 °C)		
Sample Preparation	**Sugar boiled confectionery & Lozenges** If composition of entire product is desired, grind and mix thoroughly. If product is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as completely as possible, and grind and mix each test portion thoroughly. **Chewing gum and bubble gum** Cut into small bits/ pieces around 50-75 g and mix well. Stored in an airtight container.		
Method of analysis	1. Place 50 g test portion in 400 mL beaker. 2. Add 50 mL mixture of Benzene and absolute alcohol (1+1), and cover with watch-glass. Heat to boiling point on steam bath, and simmer few min, stirring occasionally.		

	3. Decant liquid into tarred, round 100 mL glass dish with flat bottom approximately 7 cm diameter. 4. Extract once more with benzene-alcohol mixture, and finally rinse with two 25 mL portions of absolute alcohol, simmering and stirring each time. With moist sugar candy, avoid over heating to prevent pieces from sticking together. Add each extract to glass dish previously placed on steam bath. 5. Evaporate until alcohol is just removed, rotating dish as it goes to dryness in order to spread extract uniformly on the bottom surface. Avoid baking shellac on dish. If fat appears to be present, wash with three 15 mL portion of petroleum ether, stirring and warming. Decant through rapid filter. Remove the solvent by evaporation and residue obtained. 6. Add mixture of 25 mL iso-amyl alcohol (B.P. – 129-132 °C) and 25 mL benzene to filter (residue), and filter back to dish. 7. Heat on steam bath with stirring, cool somewhat, and transfer solution with suspended matter to 125 mL separator and rinse dish with 25 mL hot (approximately 60 °C) water, and add to separator; shake well and filter and wash water if necessary. 8. Repeat washings with water twice (or until washings are colourless), rinsing dish well around sides with first portion of liquid. 9. Finally, filter solution of shellac into tared dish (W), rinsing separator and filters with little absolute alcohol. 10. Evaporate to dryness on steam bath, rotating dish to give uniform film. 11. If much fat was extracted in original benzene extraction, wash final shellac residue with 25 mL petroleum ether, warming and stirring. Decant, dry on steam bath and in 100 °C oven and weigh. 12. After weighing, check for complete removal of sugars by thoroughly rinsing dish and surface of shellac with hot water, warming on steam bath, decanting, rinsing down with alcohol and evaporating with care to give uniform film on dish. 13. Dry and reweigh (W_1).
Calculation with units of expression	% Shellac = $\frac{(W_1\text{-}W) \times 100}{M}$

	W = weight of the empty flask W_1 = weight of the flask with shellac M=weight of the sample taken
Inference (Qualitative Analysis)	NA
Reference	A.O.A.C 21st edn, Official Method of Analysis (2019) Method no.949.11 Sugar and sugar products.
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.027:2024	**Revision No. & Date**	0.0
Scope	Chocolate		
Caution	Wear gloves during analysis		
Principle	Chocolate coating Scraped and filling of chocolate separated. Component of Filled Chocolate obtained after weighing chocolate filling.		
Apparatus/Instrument	General Apparatus and Glassware (Page 3 and 4). 1. Weighing balance. 2. Dish.		
Preparation of Reagents	NA		
Sample Preparation	**Chocolate** 1. Melt the product in a beaker at a temperature of 45-50 °C. Pour the melted sample on a marble slab and mix thoroughly with a spatula till the product is solidified and transfer to a stoppered glass bottle. Store in a cool place. 2. Chill the material until hard and then grate or shear to a fine granular condition. Mix thoroughly and transfer to a stoppered glass bottle. Store in a cool place. 3. Alternatively melt in a suitable container by placing container in water bath at about 50 °C. Stir frequently until test portion melts and reaches temperature of 45 – 50 °C, remove from bath, stir thoroughly and while still hot remove test portion for analysis using glass or metallic tube provided with close fitting plunger to expel test portion from tube or disposable plastic syringe.		

Method of analysis	1. Weigh to the nearest 0.1 g, 500 g of the filled chocolate. 2. Scrape the chocolate coating and separate the filling. Filling should not be included in the analysis. 3. Weigh the filling to the nearest 0.1 g. $\text{Chocolate component, \% by mass} = \frac{(M1 - M2) \times 100}{M1}$ Where, M1 = mass in g, of the filled chocolate taken for test M2 = mass in g, of the filling
Inference (Qualitative Analysis)	NA
Reference	IS: 1163-2023 Specification for chocolates
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Method No.	FSSAI 04C.028:2024	**Revision No. & Date**	0.0
Scope	Chocolate		
Caution	Wear gloves and face protection while doing analysis.		
Principle	Liquefied sample is drained through 20 mesh sieve and retained residue is calculated as edible whole substance after drying.		
Apparatus/Instrument	General Apparatus and Glassware 1. Weighing Balance 2. Glass /Metal container. 3. Sieve 20 mesh. 4. Tray.		
Materials and Reagents	1. Trichloroethylene		
Sample Preparation	**Chocolate** 1. Melt the product in a beaker at a temperature of 45-50 °C. Pour the melted sample on a marble slab and mix thoroughly with a spatula till the product is solidified and transfer to a stoppered glass bottle. Store in a cool place. 2. Chill the material until hard and then grate or shear to a fine granular condition. Mix thoroughly and transfer to a stoppered glass bottle. Store in a cool place. 3. Alternatively melt in a suitable container by placing container in water bath at about 50 °C. Stir frequently until test portion melts and reaches temperature of 45 – 50 °C, remove from bath, stir thoroughly and while still hot remove test portion for analysis using glass or metallic tube provided with close fitting plunger to expel test portion from tube or disposable plastic syringe.		
Method of analysis	7. Weigh to the nearest 1.0 g, 500 g of the product containing fruits, nuts etc. 8. Break the sample into small pieces and place them in 1 L glass/ metal container. 9. Cover the sample with melted cocoa butter and place container in a warm oven until the added ingredients can be separated upon stirring. 10. Sieve contents through a 20 mesh sieve and allow the liquid to drain		

	completely. 11. Next soak the sieve containing ingredients in trichloroethylene and stir gently for a minute or two. 12. Remove cleaned nuts, fruits etc, onto a tray and let the solvent evaporate. Weigh to the nearest 0.1 g.
Calculation with units of expression	Wholesome ingredients,% by mass = $\frac{\text{Mass of residue x 100}}{\text{Sample wt.}}$
Inference (Qualitative Analysis)	NA
Reference	IS: 1163-2023 Specification for chocolates
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Method No.	FSSAI 04C.029:2024	**Revision No. & Date**	0.0
Scope	Chewing Gum And Bubble Gum		
Caution	Concentrated sulphuric acid is corrosive, causes burns. Wear mask and gloves during analysis.		
Principle	Titanium oxide is estimated using spectrophotometer.		
Apparatus/Instrument	General Apparatus and Glassware 1. Beaker 2. Glass beads 3. Watch glass 4. Steam bath 5. Volumetric flasks 6. Weighing balance 7. Silica dish 8. Burner 9. Muffle furnace 10. Hot plate 11. Graduate cylinders 12. Spectrophotometer 13. Volumetric flasks		
Materials and Reagents	(1) Titanium di-oxide (2) Anhydrous sodium sulphate – Analar grade (3) Conc. Sulphuric acid. (4) Distilled water. (5) Hydrogen peroxide – 30% grade		
Preparation of Reagents	1. Titanium di-oxide standard solution: (0.1 mg/mL) - Weigh accurately 50 mg titanium dioxide in a 250 mL beaker. Add 15 g anhydrous sodium sulphate and 50 mL conc. Sulphuric acid. Add 1 or 2 glass beads, cover with watch glass and heat on a hot plate to boil and dissolve. Cool and add 100 mL distilled water accurately with stirring. (If the solution is cloudy warm on a steam bath to clarify) Cool and transfer to 500 mL volumetric flask containing 200 mL		

	water. Make up to volume. 2. Dilute Sulphuric acid: Concentrated sulphuric acid is diluted ten times by mixing with distilled water.
Sample Preparation	Cut into small bits/ pieces around 50-75 g and mix well. Stored in an airtight container.
Method of analysis	1. Accurately weigh 2-3 g of prepared sample in a 100mL silica dish. 2. Char the material on a burner and ash in muffle furnace at 800 °C for 3-4 h. 3. Cool, add 2g anhydrous sodium sulphate and 10 mL conc. H_2SO_4, cover with watch glass and bring to boiling on a hot plate and dissolve. 4. Cool thoroughly and rinse the watch glass carefully with 30 mL water. Transfer to 100 mL volumetric flask. (If solution is cloudy heat on steam bath to clarify) cool and dilute to volume with water. 5. Transfer 3 mL aliquot of the sample solution to 5 mL volumetric flask or graduated cylinder. Dilute to volume with 10% H_2SO_4. Add 0.2 mL 30% H_2SO_4. Mix well. 6. Measure the absorbance at 408 nm against a prepared blank. Determine the concentration of TiO_2 in sample using a standard curve. **Preparation of Standard Curve:** 7. Transfer 0, 1, 2, 3 and 4 mL of TiO_2 standard solution (0.1 mg/mL) to 5mL volumetric flask or graduated cylinder. 8. Dilute to volume with 10% H_2SO_4. 9. Mix well. Measure the absorbance of the colour at 408 nm in a spectrophotometer and prepare a standard curve.
Calculation with units of expression	TiO_2 (in g %) = $\frac{\text{Dilution x mg } TiO_2 \text{ x Dilution x Absorbance of sample x } 100}{\text{Wt. of sample x Dilution x Absorbance of standard x } 1000}$
Inference (Qualitative Analysis)	NA
Reference	A.O.A.C 21st edn, Official Method of Analysis (2019) Method no. 973.38 Titanium Dioxide in Cheese.
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Method No.	FSSAI 04C.030:2024	**Revision No. & Date**	0.0
Scope	Chewing Gum And Bubble Gum		
Caution	Wear mask and gloves during handling of solvent		
Principle	Gums are extracted using a solvent, where gum-base is soluble (viz., chloroform). Gum-base is determined after removal of solvent.		
Apparatus/Instrument	General Apparatus and Glassware (Page 3 and 4) (1) Soxhlet extraction apparatus (2) Whatman thimble. (3) Weighing balance. (4) Hot air-oven. (5) Desiccator.		
Materials and Reagents	1. Chloroform, analytical grade. 2. Desiccants for Dessicator		
Sample Preparation	Cut into small bits/ pieces around 50-75 g and mix well. Stored in an airtight container.		
Method of analysis	1. Weigh 4-5 g of sample (W) in a Whatman thimble and extract the gum in a continuous extraction apparatus (Soxhlet extractor) with chloroform for 8 h. 2. Distil off or evaporate the chloroform extract on a steam bath and transfer the flask to an oven maintained at 100 °C and dry it for 4-5 h. 3. Cool in a desiccator and weigh (W2). 4. Chloroform extract must be dried by keeping the flasks for 30 min and weighed, till constant mass is achieved.		
Calculation with units of expression	Gum base Content % = $\frac{((M1 \times M2)}{M} \times 100$ Where M1 = mass in g of the flask with extracted gum sample M2 = mass in g of the empty flask M = mass in g of the sample taken for test		
Inference (Qualitative Analysis)	NA		
Reference	IS: 6747-2018 Chewing gum - Specification		
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Method No.	FSSAI 04C.031:2024	**Revision No. & Date**	0.0
Scope	Cocoa mass or cocoa/chocolate Liquor and cocoa cake		
Caution	Wear mask and gloves during analysis		
Principle	Determination of Cocoa shell is based on sample preparation followed by Microscopic examination		
Apparatus/Instrument	General Apparatus and Glassware 1. Sieve: - No. 230, 5 in. (13cm) diameter, stainless steel. 2. Grinding equipment:- (1) Coarse grinding (cutting action) (2) Fine grinding: - 13 cm (5 in.) glass mortar and pestle or. electric mortar grinders MG1 or MG2. Adjust MG2 so that pestle and shaft are not under tension by loosening top knob and lock nut by three turns and adjust closing spring control to ½ tension. 3. Aluminum Dish: - Diameter ca 77 mm, height ca 33 mm; with cover. 4. Brush: - No. 10, nylon, rubber set, oval sash paint brush with bristles cut to 4-4.5 cm		
Materials and Reagents	NA		
Sample Preparation	(a) Chocolate liquor, chocolate: - (1) Chill 200 g sweet or bitter chocolate until hard, and grate or shave to fine granular condition. Mix thoroughly and preserve in tightly stoppered bottle in cool place. Alternatively, (2) Melt 200 g bitter, sweet, or milk chocolate by placing in suitable container and partly immersing container in bath ca 50 ^{o}C. Stir frequently until test portion melts and reaches temperature of 45-50 ^{o}C. Remove from bath, stir thoroughly, and while still liquid, remove test portion for analysis, using glass or metal tube, 4-10 mm diameter, provided with close- fitting plunger to expel test portion from tube, or disposable plastic syringe. (b) Expeller cake :- Crush with mortar and pestle and grind to pass No. 30 sieve in mill, (b) (1), Ca ½ teaspoonful at time. Mix well and store in tightly stoppered jar. (c) Cocoa press cake:- Prepare and store as in (b) (Many test samples can be easily pulverized after drying 2-3 h at 60-70 ^{o}C) (d) Cocoa:- Use as is. Store as in (b).		

	Defatting and Grinding • Set up sieve in 15 cm (6 in.) glass funnel with tip dipping 2 cm into 500 ml flat- bottom Pyrex centrifuge bottle. • Place 15 g cocoa, coarsely ground (30-40 mesh) cocoa press cake, or expeller cake, or 25-30 g chocolate or chocolate liquor in 250 ml centrifuge bottle. Add 100 ml ether, stopper, shake thoroughly to dissolve fat, and pour onto sieve. Wash material on sieve well with ether. Let material on sieve stand until dry. • Centrifuge mixture in 500 ml centrifuge bottle 10 min at 2000 rpm. Decant and discard supernate. Replace centrifuge bottle under funnel. • Place sieve with dried cocoa material in receiver (sieve bottom pan). Brush material through sieve with No. 10 sash paint brush. Transfer retains, using brush, to 12.7 cm glass mortar and grind 45 s with glass pestle, or grind 2 min in motor-driven mortar grinder. Transfer to sieve and rebrush. • Repeat grinding and brushing until virtually all material passes through sieve. Quantitatively transfer material, including small amount on sieve (< 20 mg), through funnel to the 500 ml centrifuge bottle. • Clean with brush, and clean brush against rim of sieve. Wash screen, receiver, mortar and pestle, and funnel (but not brush) with ether, letting washing run into centrifuge bottle. • Rub off coated material on funnel and other apparatus with policeman, rinsing with ether through funnel into centrifuge bottle. Stopper bottle and shake thoroughly. • Remove stopper and rinse with ether. Centrifuge 10 min at 2000 rpm. Decant and discard supernate. Add 100 ml ether and repeat extraction. Add 100 ml ether, Stopper, and shake. Immediately pour into fritted glass crucible (disk diameter 60 mm ; medium porosity) under vacuum. Wash material from bottle into crucible with ether. Wash twice with ca 35 ml ether and continue vacuum until dry (20 min).

	• Quantitatively transfer material from crucible to glass mortar and grind gently until fine. (Spoon may be used in transfer but used in transfer but use rubber policeman to scrape disc). • Quantitatively transfer ground material to Al dish. With cover in place, rotate dish until contents are well mixed. Dry on steam bath 10-15 min to remove traces of ether, and then in oven 1 h at 100 ^{o}C.
Method of analysis	• Make duplicate determination. Accurately weigh 0.350 g extracted and dried material and transfer to 150 ml beaker. Gradually stir in 25 mL 4% NaOH solution (w/v) until smooth. Bring to initial boil, using electric hot plate. • Immediately reduce to low heat and boil gently 2 min with frequent stirring. Cool somewhat and transfer to 25 x 100 mm pyrex culture tube with small portions H_2O. • Centrifuge until clear (3 min) at full speed of International clinical centrifuge, using No. 571curved rubber cushion in No. 320 shield, or equivalent. Decant carefully and discard supernate. Add H_2O to tube until ¾ full, stopper, and shake until residue is well dispersed. • Centrifuge and decant as before. Add H_2O to tube until ½ full, stopper, and shake until product is well dispersed. Transfer solution to 50 ml glass-stoppered graduate containing 25 ml glycerol. • Wash remaining material from tube to graduate with small portions H_2O, stoppering and shaking tube to aid transfer. Dilute to 50 ml with H_2O, and shake. Transfer to 100 ml beaker. • Stir well with vertical rotary motion. While stirring, withdraw small drop to Howard mold counting chamber, and make slide • Clean Howard cell and cover with cover glass so that Newton's rings are produced between slide and cover glass. Remove cover and with Knife blade or scalpel, place portion of well-mixed test sample on central disk; with same instrument, spread evenly over disk, and cover with glass so as to give uniform distribution. Discard any mount showing uneven distribution or absence of Newton's rings, or liquid that has been drawn across moat and between cover glass and shoulder.

- With microscope adjusted for mold counting (field of view 1.382 mm at 100X), count fields positive for spiral vessel, at varying depths, at 200X in 25 fields of each of eight slides of each of the two determinations (total of 400 fields). Report as positive field one that contain any portion of section of spiral vessels, but none smaller than well developed “S” or “Z” either separate or attached to piece of shell. Average results and report as % positive field present. This is spiral vessel count.

Counting Instructions

- Spiral vessel varies greatly in size. No distinction is made in counting because of size differentiation. In appearance spiral vessels have parallel walls of even intensity with clear centers. On occasionally piece, walls may be frayed due to grinding. Walls of very small vessels do not appear as sharp as those of the larger ones at 200x. Some spirals are closely knit together. Photomicrographs of spiral vessels and positive sections of them are shown in Figure 4.

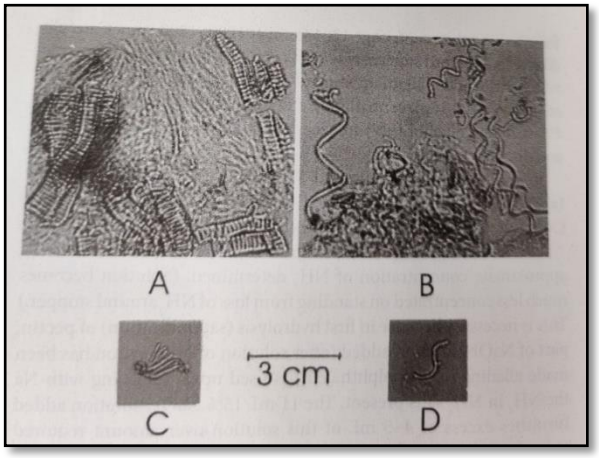

Figure 4. Spiral vessels sections ca 340x. (A) Mass of spiral vessels; note size differential and narrowing effects. (B) Spiral vessels stretched out; note difference in size. (C) Broken section; count positive if three joined rods are present. (D) Positive “S” shaped section.

- In counting spiral vessels, most positive fields counted will have easily recognized positive spiral vessel figures such as long or short mass of spiral vessels, large broken sections of these, or sometimes tangled mass

of spiral vessels. Some sine wave-like figures and some full "S" or "Z" like figures will be found. There will, however, be some smaller figures and poorly formed "S" or "Z" like figures present in some fields. For these figures, the following applies:

"S" or "Z" like figures or mirror images of these should have 1/3 or more of top and bottom normal linear distance for such figure and not just stubs. One-third of centre section is estimate of this distance. If "2" like figure is found, lower portion or "V" part of figure should be well extended and sufficient top curve should be present so that figure does not appear essentially like a "V". Figures may be stretched out. Spiral vessels in breaking sometimes break so that there will be joined sections of half circles or less. Count as positive any such section consisting of ≥3 nearly ½ circles joined. Two spiral circles joined together are counted positive. Circles showing no spiraling are not counted. A "W" figure is positive; a "V" or "C" is not. In viewing small section of spirals perpendicular to axis, second and third spiral, etc., may be just faintly seen, but the section should be counted as positive.

- There is some fine cell wall structure present which when broken may fracture into "Z" like characters similar in appearance to "Z" formed from small thin spiral vessels. Care should be exercised in discriminating between the two.
- Determine % shell in chocolate component by comparison with standard curve prepared from spiral vessel count values listed in Table (A) plotted against % shell in chocolate component. Use column for counts listed under "≤ 15% shell".

Table A: Standard spiral vessel count values

Spiral Vessel Count		
Shell in chocolate Component %	≤15% shell (0.350 g/ 50 ml)	≥15% shell (0.200 g/ 100 ml)
0	4.5	1.5
1	15	5.8
2	24.4	9.7

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<tr><td>4</td><td>40</td><td>16.6</td></tr>
<tr><td>5</td><td>47</td><td>19.7</td></tr>
<tr><td>8</td><td>62.2</td><td>27.7</td></tr>
<tr><td>11</td><td>72.9</td><td>34.8</td></tr>
<tr><td>15</td><td>83.4</td><td>42.4</td></tr>
<tr><td>20</td><td>91.1</td><td>50.1</td></tr>
<tr><td>30</td><td>98.2</td><td>62.1</td></tr>
<tr><td>60</td><td></td><td>80.0</td></tr>
<tr><td>100</td><td></td><td>86.8</td></tr>
</table>
</td></tr>
<tr><td>Calculation with units of expression</td><td>Spiral Vessel count Values
For 1-15 % shell (spiral vessel counts of 15-83.4), following formula gives values comparable to Table A
S = 538P-1777/ 7043-50P
Where S= Shell in chocolate component and P = spiral vessel count.
For test samples containing 15 % shell (spiral vessel count > 83.4) repeat determination throughout, but weigh 0.200 g test portion and dilute to 100 ml with H_2O in 100 ml glass stoppered graduate containing 50 ml glycerol. Count at 200X. Use column for counts listed under “ > 15% shell” for preparing standard curve.</td></tr>
<tr><td>Inference (Qualitative Analysis)</td><td>NA</td></tr>
<tr><td>Reference</td><td>AOAC Official Method 968.10 (2023)</td></tr>
<tr><td>Approved by</td><td>Scientific Panel on Methods of Sampling and Analysis</td></tr>
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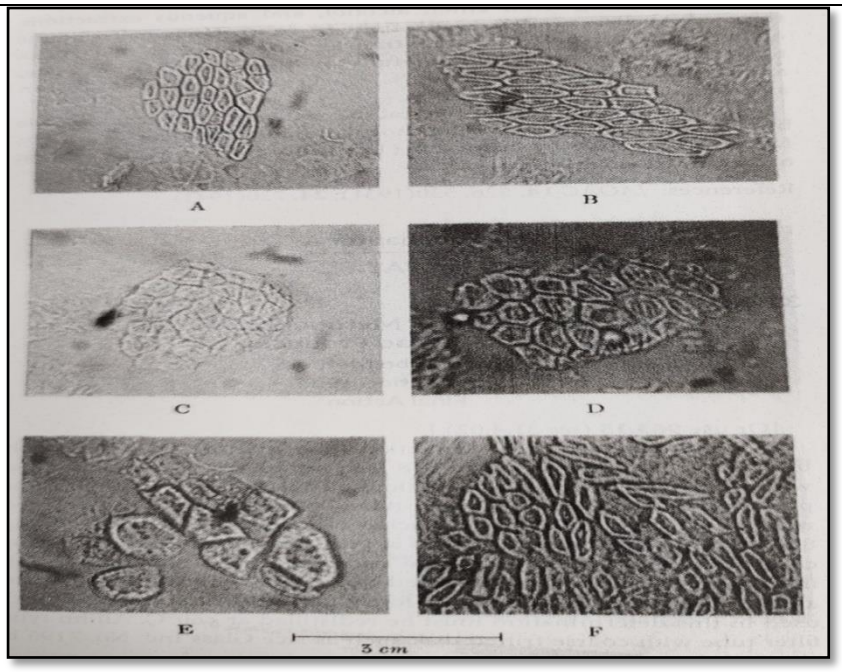
एफएसएसएआई fssai भारतीय खाद्य सुरक्षा और मानक प्राधिकरण Food Safety and Standards Authority of India स्वास्थ्य और परिवार कल्याण मंत्रालय Ministry of Health and Family Welfare	**Determination of Cocoa Shell and Germ % on Alkali free Basis in Cocoa mass or cocoa/chocolate Liquor and cocoa cake**		
Method No.	FSSAI 04C.032:2024	**Revision No. & Date**	0.0
Scope	Cocoa mass or cocoa/chocolate Liquor and cocoa cake		
Caution	Wear mask and gloves during analysis		
Principle	Determination of Cocoa shell is based on sample preparation followed by Microscopic examination		
Apparatus/Instrument	General Apparatus and Glassware 1. Sieve: - No. 230, 5 in. (13cm) diameter, stainless steel. 2. Grinding equipment:- (1) Coarse grinding (cutting action) (2) Fine grinding: - 13 cm (5 in.) glass mortar and pestle or electric mortar grinders MG1 or MG2. Adjust MG2 so that pestle and shaft are not under tension by loosening top knob and lock nut by three turns and adjust closing spring control to ½ tension. 3. Aluminum Dish: - Diameter ca 77 mm, height ca 33 mm; with cover. 4. Brush: - No. 10, nylon, rubber set, oval sash paint brush with bristles cut to 4-4.5 cm 5. Slide and cover glass: - 75 X 38 mm slide with lines 0.5 mm apart, nearly across slide. Parallel to 75 mm side, and ruled from top to bottom; 33x33x0.2 mm cover glass. 6. Scoop:- Thin (0.01-0.02 mm thick) stainless steel strip ca 4.8 mm wide with 90^{o} bend extending outward 3 mm. 7. Magnetic stirrer:- With stirring bar ca 16 long X 6 mm diameter. Stirring bar 13 X 8 mm with ridge in center will circle walla of 1 oz ointment jar (36 mm diameter X 40 mm high internal measurements) with distinct convex bottom, giving both vortex mixing and stirring.		
Materials and Reagents	1. Bellucci's reagent :- CH_3COOH-H_2O-HNO_3 (36+9+5)		
Sample Preparation	**Defatting and Grinding** • Set up sieve in 15 cm (6 in.) glass funnel with tip dipping 2 cm into 500 ml flat- bottom Pyrex centrifuge bottle. • Place 15 g cocoa, coarsely ground (30-40 mesh) cocoa press cake, or expeller cake, or 25-30 g chocolate or chocolate liquor in 250 ml centrifuge bottle. Add 100 ml ether, stopper, shake thoroughly to dissolve fat, and pour onto sieve. Wash material on sieve well with ether. Let material on sieve stand until dry. • Centrifuge mixture in 500 ml centrifuge bottle 10 min at 2000 rpm. Decant and discard supernate. Replace centrifuge bottle under funnel.		

	• Place sieve with dried cocoa material in receiver (sieve bottom pan). Brush material through sieve with No. 10 sash paint brush. Transfer retains, using brush, to 12.7 cm glass mortar and grind 45 s with glass pestle, or grind 2 min in motor-driven mortar grinder. Transfer to sieve and rebrush. • Repeat grinding and brushing until virtually all material passes through sieve. Quantitatively transfer material, including small amount on sieve (< 20 mg), through funnel to the 500 ml centrifuge bottle. • Clean with brush, and clean brush against rim of sieve. Wash screen, receiver, mortar and pestle, and funnel (but not brush) with ether, letting washing run into centrifuge bottle. • Rub off coated material on funnel and other apparatus with policeman, rinsing with ether through funnel into centrifuge bottle. Stopper bottle and shake thoroughly. • Remove stopper and rinse with ether. Centrifuge 10 min at 2000 rpm. Decant and discard supernate. Add 100 ml ether and repeat extraction. Add 100 ml ether, Stopper, and shake. Immediately pour into fritted glass crucible (disk diameter 60 mm; medium porosity) under vacuum. Wash material from bottle into crucible with ether. Wash twice with ca 35 ml ether and continue vacuum until dry (20 min). • Quantitatively transfer material from crucible to glass mortar and grind gently until fine. (Spoon may be used in transfer but used in transfer but use rubber policeman to scrape disc). • Quantitatively transfer ground material to Al dish. With cover in place, rotate dish until contents are well mixed. Dry on steam bath 10-15 min to remove traces of ether, and then in oven 1 h at 100 ^{o}C.

Method of analysis	• Mix dried (1 h at 100°C) product by tumbling in covered dish. Make duplicate determinations. Accurately weigh 0.500 g extracted and dried material and transfer to 150 ml beaker. Gradually stir in a portion of 20 ml Bellucci's reagent until smooth; rinse walls of beaker and stirring rod with remainder. Stir gently. Fill short-neck, 100 ml, round-bottom flask with cold water to neck and place on top of beaker; let rod rest in spout of beaker. Bring solution to initial boil, using electric hot plate. Immediately reduce to low heat and boil gently 10 min with frequent gentle swirling, keep trig beaker and flask together. Cool ca 5 min. • Accurately weigh 25 X 100 mm Pyrex, rimless culture tube in 30 ml beaker (holder). Quantitatively transfer residue to culture tube with small portions H_2O, scrubbing beaker and rod with rubber policeman. Centrifuge ≥3 min at full speed in international clinical centrifuge, using IEC No. 571 curved rubber cushion in IEC No. 320 shield, or equivalent. Decant carefully and discard supernate (some flocculent material may present). Add H_2O to tube to ca ¾ full, stopper, and shake until residue is well dispersed. Remove stopper, rinse, centrifuge, and decant as before. • Add aqueous glycerol (3+2) to culture tube until tube and holder weigh 20 ± 0.03 g more than original weight. Stopper, shake vigorously until well mixed, and transfer immediately to 1 oz ointment jar containing small magnetic bar. Stopper jar and let stand until bubbles disappear (ca 5-10 min). • Accurately weigh together ruled glass slide and cover glass. Stir liquid in jar 1 min on magnetic stirrer at maximum speed at which small bubbles do not form. Stop. In rapids sequence, push jar (to put magnetic bar next to wall of jar) and, using scoop, immediately transfer drop liquid (ca 0.04 ± 0.01 g) to centre of tared slide, rulings up. Tap slide gently with scoop several times to remove as much liquid as possible. Place cover slip so that one edge rest just above and parallel to lower edge of slide. Lower cover slip carefully until it touches liquid and then let it drop. Liquid will ooze to edges. Do not press cover slip. Weigh prepared slide to 0.1 mg. Place rubber stopper in jar to prevent

	evaporation. • Place slide on compound microscope with or without upper half of condenser and with transmitted day-light-type filtered and diffused light. Count two slides from each of two determinations as in (a) or (b) : (a) Stone cell count: - For cocoa, cocoa press cake, chocolate liquor, and expeller cake. Scan slide at 100X and count stone cells at ≥ 200 X. Count whole stone cells, both single and in groups, and all broken stone cells which are ≥0.5 cell. Do not count smaller fragments. (b) Stone cell group count: - For other chocolate products. Proceed as in (a), counting only stone cell groups containing ≥2 stone cells. **Description of stone cells** Stone cells vary considerably in size, shape, and general appearance. Some are very distinct and others are relatively indistinct. Their size varies from ca 10 to 38 µm; the longest are very slender. Some very coarse stone calls up to ca 40 µm with thick, beaded appearing outside wall ca 7 µm wide are occasionally found. Stone shapes are polygonal, generally irregular, and may contain curved areas. On well-developed stone cells outside walls are 2-3.5 µm wide. On less distinct stone cells, outside walls are narrower and thinner; such cells are immature or not fully developed. Several near-parallel thin walls or lines, viewed microscopically, are easily visible in many stone cells. They are generally more distinct in those where outside wall is thin. See Figure 1. For photomicrographs of stone cells. Stone cells usually are in group formation, consisting of ≥2 stone cells.

	 **Figure 1:-** Stone cells ca 330x: (A) and (B), distinct stone cells; (C) indistinct stone cells; (D) stone cells showing distinct parallel lines in central area; (E) very large stone cells infrequently found; (F) long stone cells attached to a large piece of shell, showing separations between stone cells on shell.
Calculation with units of expression	For either method, average four S values from one of formulas below and report as % shell in chocolate component: (a) Stone cell count:- $S_1 = 84C/17200M – C$ (b) Stone cell count:- $S_2 = 84C/17200M – C$ Where W = g test portion; L = g diluted test solution; D = g of drop counted; C = stone cell count of drop; M = mg dry, fat- free test portion in drop counted (= 1000 WD/L); S = % shell in chocolate component; G = stone cell groups in drop; and 9340 = number stone cells in 1 mg dry, fat free, 250 mesh shell. Example: For 0.5 g test portion diluted to 20 g, $S_1 = 84C/(430000D – C)$ and $S_2 = 84G/(42500D – G)$.
Inference (Qualitative Analysis)	NA
Reference	AOAC Official Method 970.23 (2023)
Approved by	Scientific Panel on Methods of Sampling and Analysis

PART B: SWEETENING AGENT

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Method No.	FSSAI 04C.033:2024	**Revision No. & Date**	0.0
Scope	Sugar & sugar products (White sugar, Refined sugar, Khandsari sugar, Cube sugar, Icing sugar, Gur or Jaggery and Cane Gur/Cane Jaggery)		
Caution	Sugars contain and absorb moisture. Once sample is opened, seal it in airtight manner after taking test portion		
Principle	Sugar sample is heated under controlled conditions to remove moisture. Sample is weighed before and after drying and the difference in sample weight before and after drying is calculated.		
Apparatus/Instrument	General Apparatus and Glassware 1. Aluminium dish with lid 2. Weighing balance 3. Oven 4. Stop Clock 5. Desiccators		
Materials and Reagents	1. Desiccants for desiccator		
Sample Preparation	Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container.		
Method of analysis	**Method A** 1. Transfer 5 g of the prepared sample in a previously dried, tared aluminium dish. 2. Cover the dish with the lid and weigh accurately. Remove the lid and dry the sample at 105 °C ± 1 °C for 3 h. 3. Cool in desiccators and weigh. 4. Re dry for 1 h and repeat process until change in weight between two successive dryings is less than 2 mg. 5. Report the loss in weight as moisture. 6. The weight difference of the sample before and after drying is moisture.		

	Method B 1. Carry out the determination in duplicate and preheat the oven to 105°C. Place the empty dishes with lids open in the oven for not less than 30 min. 2. Remove the dishes from the oven, close the lids and place in the desiccator. Place the contact thermometer (probe) on top of one of the dishes. 3. When the temperature of the dishes has fallen to ambient + 2°C, weigh them as rapidly as possible to an accuracy of $\pm$ 0.1 mg (m_1). 4. As rapidly as possible, place 20 to 30 g. of the sample into each dish, close the lids and weigh the dish on balance (m_2). 5. Return the dishes with the lids open to the oven. Dry the sample for 3 h at 105°C$\pm$ 1 °C. Ensure that there are no other materials in the oven during the drying period. 6. Close the lids; remove the dishes from the oven and place in the desiccator with the contact thermometer (probe) on one of them. Cool the dishes until the thermometer indicates a temperature of ambient + 2°C. Weigh the dishes (m_3). **Note**: - The ICUMSA method (2007) requires a forced draft oven maintained at a temperature of 105 ± 1°C. The oven is to be ventilated and the circulation fan fitted with an interlock switch which opens when the oven door is opened the dishes with tight fitting lids should be 6-10 cm with a depth of 2-3 cm although the dish may be made of glass, platinum or Nickel, aluminium is recommended. The quantity of sample recommended is 20 – 30 g, the depth of sample in the dish not to exceed 1 cm.
Calculation with units of expression	Moisture (%) (by weight) $= \frac{W_1 - W_2}{W1 - W} \times 100$ Where, W = Weight in g, of Aluminium dish. W_1 = Weight in g, of Aluminium dish + sample before drying. W_2 = Weight in g, of Aluminium dish + dried sample.

	Results are acceptable if deviation between duplicate values is not outside the limit of ± 10% of the mean value for the test. If deviation exceeds this limit the test should be repeated. $$\text{Deviation (\%)} = \frac{\text{(one of the value of duplicate – Average value) x 100}}{\text{Average value}}$$
Inference **(Qualitative Analysis)**	NA
Reference	• A.O.A.C 21st edn, Official Method of Analysis (2019) Method no. 925.45 (b) (except 105 °C temperature as per P.F.A) Moisture in Sugars. • IS 15729:2011 Sugar and sugar products • ICUMSA GS2/1/3/9 -15 (2007)
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.034:2024	**Revision No. & Date**	0.0
Scope	Cane sugar and refined sugar, Cube sugar, Misri, Gur or jaggery		
Caution	Once sample is opened, seal it in airtight manner after taking test portion Wear heat resistant gloves and face protection while doing analysis		
Principle	Ash is the inorganic residue remaining after destruction of organic matter at a temperature of 550 ± 25 °C. Sample is weighed before and after heat treatment to estimate total ash.		
Apparatus/Instrument	General Apparatus and Glassware 1. Silica dish 2. Burner 3. Muffle furnace 4. Desiccator 5. Weighing balance		
Materials and Reagents	1. Desiccants for Desiccator		
Sample Preparation	Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container.		
Method of analysis	**1.** Weigh accurately 5 - 10 g of sample in a previously dried and weighed silica dish. **2.** Char the sample on a burner and transfer the dish to muffle furnace maintained at 525±25 °C. Cool in desiccator and weigh. Incinerate to a constant weight and calculate the % ash.		
Calculation with units of expression	Total ash (% on dry weight) = $\frac{(W_2 - W) \times 100 \times 100}{(W_1 - W) \times (100 - M)}$ Where, W_1 = Weight in g of Silica dish. + sample W_2 = Weight in g of Silica dish + ash W = Weight in g of empty Silica dish. M = Moisture % of the sample.		
Inference (Qualitative Analysis)	NA		
Reference	A.O.A.C 21st edn, Official Method of Analysis (2019) Method no. 900.02		
Approved by	Scientific Panel on Methods of Sampling and Analysis		

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Method No.	FSSAI 04C.035:2024	**Revision No. & Date**	0.0
Scope	Khandsari sugar, Bura, Gur or Jaggery and Cane Gur/Cane Jaggery		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Concentrated hydrochloric acid is corrosive, has an irritant vapor and causes burns. Wear mask and gloves during analysis		
Principle	Total ash is dissolved in dilute hydrochloric acid and acid in-soluble ash is determined. The proportion of ash that is not hydrolyzed by acid is known as the acid insoluble ash (silica and oxalates). The sample is ashed at a temperature 550°C ± 20 and the residue weighed.		
Apparatus/Instrument	General Apparatus and Glassware 1. Silica dish. 2. Muffle furnace. 3. Burner. 4. Filter paper - Ash less (Whatman 41or 42 or equivalent) 5. Filtration system.		
Materials and Reagents	1. Conc. Hydrochloric acid 2. Distilled Water		
Preparation of Reagents	Dilute Hydrochloric acid (Approx 5 N): Hydrochloric acid (445 mL) diluted to 1 L using distilled water.		
Sample Preparation	Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container.		
Method of analysis	1. Weigh 5 g of sample in a silica dish. 2. Char the sample and ash in a furnace at 550 °C ± 25 °C. 3. Add 25 mL of Hydrochloric acid (1:2.5) to the ash. Cover the dish with a watch glass and boil for 5 min. 4. Cool, filter through an ashless filter paper (Whatman No. 42 or 41 or equivalent) and wash the residue well with hot water until acid-free. 5. Return the filter paper to the silica dish and incinerate at 550 °C ± 20 °C. 6. Cool, weigh and calculate as %, acid insoluble ash.		
Calculation with units of expression	Ash insoluble in dilute HCl (%) (on dry wt.) = $\frac{(W_2 - W) \times 100 \times 100}{(W_1 - W) \times (100 - M)}$ Where, W_2 = weight of dish + acid insoluble ash W_1 = weight of dish + sample W = weight of empty dish M = Percent moisture		

Inference (Qualitative Analysis)	NA
Reference	IS 12924:2011 Bura Specification
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.036:2024	**Revision No. & Date**	0.0
Scope	Cane, Jaggery or Cane sugar products		
Caution	Concentrated sulphuric acid is corrosive, causes burns. Wear mask and gloves during analysis.		
Principle	Sulphated ash test utilizes a procedure to measure the amount of residual substance not volatilized from organic sample when the sample is incinerated in the presence of sulphuric acid.		
Apparatus/Instrument	General Apparatus and Glassware 1. Electric Furnace with Air Circulation, capable of being controlled at 60 to 70°C 2. Dessicator 3. Water Bath, at 60 to 70”C 4. Analytical Balance		
Materials and Reagents	1. Concentrated Sulphuric Acid 2. Desiccants for Dessicator		
Preparation of Reagents	1. Sulphuric Acid - 10 % (m/v)		
Sample Preparation	1. Mix the sample carefully and quickly by stirring (for a powder) or by mixing with a spatula (for a liquid) in a sample container. 2. If the volume of the container is insufficient for this, quickly transfer the whole sample to another, previously dried container of a suitable size. Take care to avoid any change in the moisture content of the sample. The taking of representative sample of approximately 5 g can be difficult (for example, glucose in lumps). In this case, use one of the procedures described below: a) Weigh carefully, to the nearest 0.01 g, approximately 100 g of the sample into a dry container, provided with a lid, previously tared with the lid. Add approximately 100 ml of water at 90°C, place the lid on the container and stir until the sample is completely dissolved. Allow to cool to ambient temperature and weigh to the nearest 0.01 g. b) Melt the sample in solid from by immersing it, in a container, provided with lid, in the water bath, controlled at 60 to 70”C, and placing the lid on the container. Remove the container from the water bath and allow it		

	to cool to ambient temperature, agitating frequently but without removing the lid, and then mix the condensed water with the sample.
Method of analysis	**1.** If a dilution has been carried out, take an aliquot portion of the solution obtained in sample preparation, so as to obtain a mass of sample corresponding to amass of test portion as given below. In all other cases, weigh, to the nearest 0.001 g, in the incineration dish (see 10.5.1), previously weighed to the nearest 0.0002 g, a mass of test sample in accordance with the following: Sulphated Ash Percent (m/m) — Mass of Test Portion (G) <5 — 10 >5<10 — 5 >10 — 2 **2. Pre-incineration** a. Add 5 ml of the sulphuric acid solution to the test portion or the aliquot portion b. Mix with a glass stirring rod and rinse with a little water, collecting the rinsing in the incineration dish. c. Heat the incineration dish slowly and carefully, over the electric hot-plate or gas burner or using the heating lamp, until completely carbonized (it is recommended that this be carried out under an extraction hood). **3. Incineration** **a.** Place the incineration dish in the oven, controlled at 525±25°C, and maintain this temperature until the carbon residue has disappeared. A period of 2 h is usually sufficient **b.** Allow to cool. Take up the residue with several drops of the sulphuric acid solution, evaporate on the edge of the oven and incinerate again for 0.5 h c. Place the incineration dish in the desiccator and allow it to cool to ambient temperature. d. Weigh the dish and contents to the nearest 0.0002 g. The incineration should be continued until constant mass is attained.

	e. Do not put more than four incineration dishes in the desiccator at any one time.
Calculation with units of expression	The sulphated ash, expressed as a percentage by mass of product as received, is given by the following formula: $\frac{(m_2 - m_1) \times 100}{m_O}$ The sulphated ash, expressed as a percentage in mass on the dry basis, is given by the following formula: $\frac{(m_2 - m_1) \times 100}{m_0} \times \frac{100}{100 - H}$ Where m_O = mass, in g, of the test portion, taking into account any dilution . m_1 = mass, in g, of the incineration dish before incineration m_2 = mass, in g, of the incineration residue and incineration dish after incineration, and H= moisture content of the product.
Inference (Qualitative Analysis)	NA
Reference	IS: 15279 - 2003 Sugar and Sugar Products
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.037:2024	**Revision No. & Date**	0.0
Scope	Dextrose		
Caution	Concentrated sulphuric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Sulphated ash test utilizes a procedure to measure the amount of residual substance not volatilized from organic sample when the sample is ignited in the presence of Sulphuric acid. The sample is ashed at a temperature 550°C ± 25 and the residue weighed.		
Apparatus/Instrument	General Apparatus and Glassware 1. Weighing balance. 2. Silica dish. 3. Heating system: Hot plate / Burner 4. Hood with exhaust facility. 5. Muffle furnace. 6. Exhaust hood.		
Materials and Reagents	1. Dextrose. 2. Conc. Sulphuric acid.		
Preparation of Reagents	1. Sulphuric acid (10%, v/v): Add concentrated sulphuric acid (1 mL) in distilled water (9 mL).		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, Withdraw portions for analytical determinations.		
Method of analysis	1. Weigh accurately about 10 g sample into a silica dish. 2. Add 0.5mL of conc. sulphuric acid or 5 mL of 10% (by weight) H_2SO_4. 3. Heat on hot plate or burner to carbonize the sample (perform in a hood with exhaust facility). 4. Then place the dish in the furnace heated at 550±25 °C for ashing. 5. Cool, again add 2 mL of 10%, v/v H_2SO_4. 6. Evaporate on steam bath. 7. Dry on hot plate. 8. Again ash at 550 °C to constant weight. 9. Express as % Sulphated ash.		
Calculation with units of expression	Sulphated ash (% on dry weight) = $\frac{(W_2 - W) \times 100 \times 100}{(W_1 - W) \times (100 - M)}$ Where, W_1 = Weight in g of Silica dish. + sample W_2 = Weight in g of Silica dish + sulphated ash		

	W = Weight in g of empty Silica dish. M = Moisture % of the sample.
Inference (Qualitative Analysis)	NA
Reference	I.S.I. Handbook of Food Analysis (Part II) – 1984 page 18
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.038:2024	**Revision No. & Date**	0.0
Scope	Dextrose		
Caution	Concentrated sulphuric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Sulphated ash test utilizes a procedure to measure the amount of residual substance not volatilized from organic sample when the sample is ignited in the presence of Sulphuric acid. The sample is ashed at a temperature 600°C ± 20 and the residue weighed.		
Apparatus/Instrument	General Apparatus and Glassware 1. Weighing balance. 2. Silica dish. 3. Heating system: Hot plate / Burner 4. Hood with exhaust facility. 5. Muffle furnace. 6. Exhaust hood.		
Materials and Reagents	1. Dextrose. 2. Conc. Sulphuric acid.		
Preparation of Reagents	1. Concentrated Suphuric Acid - sp gr 1.84.		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, Withdraw portions for analytical determinations.		
Method of analysis	1. Accurately weigh about 5 g of the sample into a 9 cm diameter platinum or silica dish. 2. Add a few drops (about l-5 ml) of concentrated sulphuric acid to the material in the dish. 3. Gently heat the dish on a hot-plate until the material is well carbonized, and then increase the heat until the evolution of sulphuric acid fumes ceases. 4. Ash the carbonized matter in a muffle furnace at 600 ±20°C. 5. Cool the ash and moisten it with a few drops of concentrated sulphuric acid, heat strongly on a hot-plate until sulphuric acid fumes cease to be evolved and finally ash in the muffle furnace at 600±20°C for about 2 hours.. 6. Cool in a desiccator and weigh.. 7. Heat again in the muffle furnace for 30 minutes at 600 ± 2O°C. Cool in a desiccator and weigh. 8. Repeat the process of heating in the muffle furnace for 30 minutes, cooling and weighing till the difference between two successive		

	weighing is less than 10 mg. R 9. Record the lowest mass.. 10. Express as % Sulphated ash.
Calculation with units of expression	Sulphated ash (% on dry weight) = $\frac{M\ 1x\ 100}{M2}$ Where, M_1 = mass in g of the ash M_2 = mass in g of the sample taken for the test
Inference (Qualitative Analysis)	NA
Reference	IS 874 (1992): Dextrose monohydrate
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Invert Sugar & Sucrose in White sugar, Refined sugar, Khandsari Sugar, Bura, Cube Sugar, Misri, Gur or Jaggery and Cane Gur/Cane Jaggery		
Method No.	FSSAI 04C.039:2024	**Revision No. & Date**	0.0
Scope	White sugar, Refined sugar, Khandsari Sugar, Bura, Cube Sugar, Misri, Gur or Jaggery and Cane Gur/Cane Jaggery		
Caution	Wear mask and gloves during analysis		
Principle	Sample (Sucrose) is inverted using acid and neutralized solution is titrated against Fehling solution. Subtraction of the reducing sugars provides the sucrose content		
Apparatus/Instrument	General Apparatus and Glassware 1. Volumetric flasks 2. Burette 3. Pipettes 4. Conical flasks 5. Bunsen burner 6. Wire guage 7. Weighing balance		
Materials and Reagents	1. Copper sulphate ($CuSO_4.5H_2O$) 2. Rochelle salt (potassium sodium tartrate) (K Na $C_4H_4O_6$. $4H_2O$) 3. Sodium hydroxide 4. Zinc acetate. 5. Acetic acid. 6. Potassium ferrocyanide 7. Methylene blue		
Preparation of Reagents	(1) Fehling A: Dissolve 69.28 g copper sulphate in distilled water. Dilute to 1000 mL. Filter and store in amber coloured bottle. (2) Fehling B: Dissolve 346 g Rochelle salt and 100 g NaOH in distilled water. Dilute to 1000 mL. Filter and store in amber coloured bottle. (3) Carrez 1 – Add 21.9 g Zinc acetate and 3 mL acetic acid in a 100 mL volumetric flask. Make up the volume with water. (4) Carrez 2 – 10.6% aqueous solution of Potassium ferrocyanide.		

	(5) Methylene Blue Indicator: Prepare 1% of methylene blue solution in distilled water.
Sample Preparation	Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container.
Method of analysis	**Standardization of the Fehling's Solution:** for Invert Sugar: Accurately weigh 4.75g of AR grade sucrose. Transfer to 500 ml volumetric flask with 50 ml distilled water. Add 5 ml conc. HCl and allow to stand for 24 hr. Neutralize the solution with NaOH (1 N) using phenolphthalein as end point indicator and make up to volume. Mix well and transfer 25 ml to a 100 ml volumetric flask and make up to volume (1 ml = 2.5 mg of invert sugar). Transfer to a burette having an off-set tip and titrate against Fehling's solution as described below for sample. Note the Titre = V1 = ------ ml Factor for Fehling's solution (g of invert sugar) = 0.0025 × V1 = ------ g **Procedure:** 1. Weigh exactly around 10 g of prepared sample and make upto 250 mL volume with water 2. Take an aliquot of 100 mL in a 500 mL volumetric flask and add 10 mL of HCl and let stand for 1½ days at 25 °C and above. 3. Dilute to 500 mL. Transfer an aliquot of 100 mL to a 250 mL volumetric flask, neutralize with NaOH (1N) and make up to volume and mix. 4. Take this solution in a burette having an offset tip. 5. Preliminary Titration: Pipette 5 mL each of Fehling A and B into 250 mL conical flask. Mix and add about 10 mL water and a few boiling chips or glass beads. Dispense solution. 6. Heat the flask to boiling. Add 3 drops of methylene blue indicator. 7. Continue the addition of solution drop wise until the blue colour disappears to a brick-red end point. (The concentration of the sample solution should be such that the titre value is between 15 and 50 mL). 8. Note down the titre value. 9. Final Titration: Pipette 5 mL each of Fehling A and B. Add sample

	solution about 2 mL less than titre value of the preliminary titration. Heat the flask to boiling within 3 min and complete the titration. Perform the titration in duplicate and take the average.
Calculation with units of expression	Calculate the reducing sugars % as shown below: Sugars % (as Invert Sugar) = Dilution x Factor of Fehling (in g) x 100 / Weight of sample x titre Sucrose % = [Reducing sugars % after inversion – Reducing sugars % before inversion] x (0.95) **For Bura** Invert the solution with HCl. Conduct the titration and calculate as given under Total sugars % expressed as sucrose = Total reducing sugars % x 0.95
Inference (Qualitative Analysis)	NA
Reference	A.O.A.C 21st edn, Official Method of Analysis(2021) method no. 945.66 Lane and Enon Method
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Method No.	FSSAI 04C.040:2024	**Revision No. & Date**	0.0
Scope	White sugar, refined sugar, khandsari sugar, bura, cube sugar, misri, gur or jaggery and cane gur/cane jaggery		
Caution	Wear mask and gloves during analysis		
Principle	An aqueous solution of the sugar sample (26 g, i.e the normal weight of sucrose in 100 mL water.) is polarized by means of a saccharimeter which is calibrated to read 100 °S on the 'International Sugar Scale' under specified conditions.		
Apparatus/Instrument	General Apparatus and Glassware 1. Saccharimeter, calibrated with quartz plates. Basis of calibration of 100° S points on international sugar scale is polarization of normal solution of pure sucrose (26.000 g/100 mL) at 20 °C in 200 mm tube using white light and dichromate filter defined by the commission. This solution polarized at 20 °C must give saccharimeter reading of exactly 100 °S. Temperature of sugar solution during polarization must be kept constant at 20 °C. Following rotations hold for normal quartz plate of international sugar scale: Normal Quartz Plate =100 °S = 40.690 ± 0.002 (´= 546.1 nm) at 20 °C In general make all polarizations at 20 °C. For countries where mean temperature is above 20 °C. Saccaharimeter may be adjusted at 30 °C or any other suitable temperature, provided sugar solution is diluted to final volume and polarized at this temperature. 2. Volumetric flasks 3. Filtration set		
Materials and Reagents	1. Dry basic lead acetate 2. Potassium or Sodium Oxalate		
Sample Preparation	Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container.		
Method of analysis	1. In determining polarization, use whole normal weight (26 ± 0.002 g) for 100 mL or multiple for any corresponding volume.		

	2. Bring solution exactly to mark at proper temperature and after wiping out the neck of the flask with filter paper. 3. Add minimum amount of dry basic lead acetate, shake to dissolve. Repeating addition till precipitation is complete. 4. Pour all clarified sugar solution on rapid air dry filter. 5. Cover funnel at start of filtration. 6. Reject first 25 mL filtrate and use remainder (must be perfectly clear) for polarization. In no case return whole solution or any part to filter. 7. To remove excess lead used in clarification add anhydrous Potassium or Sodium Oxalate to clarified filtrate in small amounts until test for lead in filtrate is negative, then re-filter. 8. Polarize in 200 mm tube and measure the reading. 9. Other permissible clarifying and decolorizing agents are alumina cream or concentrated alum solution.
Calculation with units of expression	Temperature correction for polarization of sugars: Polarization when made at temperatures other than 20 °C may be calculated to polarization at 20 °C by the following formula: $P_{20} = P_t [1 + 0.0003 (t - 20)]$ Where, Pt = polarization at temperature read. For Bura Invert the solution with HCl. Conduct the titration and calculate as given under Total sugars % expressed as sucrose = Total reducing sugars % x 0.95
Inference (Qualitative Analysis)	NA
Reference	A.O.A.C 21st edn, Official Method of Analysis(2019) method no. 925.46 Sucrose in Sugars and syrups Polarimetric method.
Approved by	Scientific Panel on Methods of Sampling and Analysis

	Determination of Sucrose in Cane sugar and refined sugar, Khandsari Sugar, Bura, Cube Sugar, Misri, Gur or Jaggery and Cane Gur/Cane Jaggery		
Method No.	FSSAI 04C.041:2024	**Revision No. & Date**	0.0
Scope	Cane sugar and refined sugar, Khandsari Sugar, Bura, Cube Sugar, Misri, Gur or Jaggery and Cane Gur/Cane Jaggery		
Caution	Concentrated Hydrochloric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Difference in the reading of polarimeter before and after polarization of sugar solution is used to determine sucrose quantity.		
Apparatus/Instrument	General Apparatus and Glassware 1. Volumetric flasks -100 mL 2. Pipettes – 50 mL 3. Water bath with heating system. 4. Polari meter. Polari meter: graduated in International Sugar Scale and provided with a 200 mm tube. It should be sheltered in a cabinet, the inside of which is maintained at 20 °C. Standardization of Polarimeter scale — Polarimeter scale must be graduated in conformity with International Sugar Scale adopted by ICUMSA. Rotations of this scale are designated as degrees sugar (^{0}Z). If the scale gives reading in ^{0}S, then to convert values in ^{0}S to values in ^{0}Z, multiply the ^{0}S value by the factor 0.99971. Basis of calibration of 100^0 point on international sugar scale is polarization of normal solution of pure sucrose (26.000 g/100 mL) at 20 °C in 200 mm tubes. This solution, polarized at 20 °C, must give Polarimeter reading of exactly 100 °Z.		
Materials and Reagents	1. Sodium chloride. 2. Distilled water. 3. Hydrochloric acid (Sp. gravity -1.109).		
Sample Preparation	Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar.		

	Transfer to a dry stoppered container.
Method of analysis	**Direct reading** 1. Pipette 50 mL lead free filterate into 100 mL volumetric flask. 2. Add 2.315 g NaCl and 25 mL water . 3. Dilute to volume with water at 20 °C 4. Polarize in 200 mm tube at 20 °C. 5. Multiply reading by 2 to obtain direct reading. **Invert Reading** **1.** Pipette 50 mL aliquot lead free filtrate into100 mL volumetric flask. **2.** Add 20 mL water. Add little by little 10 mL HCl (sp. gravity 1.109) while rotating flask. **3.** Heat water bath and maintain at 60 °C. **4.** Place flask in water bath, agitate continuously for 3 min and leave flask in bath exactly 7 min longer. **5.** Place flask at once in water at 20 °C. **6.** When contents cool to 35 °C **7.** Leave flask in bath at 20 °C at least 30 min longer and finally dilute to mark. **8.** Mix well and polarize in 200 mm tube provided with lateral branch and water jacket, keeping temperature at 20 °C. **9.** Multiply by 2 obtain invert reading. **10.** If it is necessary to work at temperature other than 20 °C which is permissible within narrow limits, volume must be completed and both direct and invert polarization must be made at exactly same temperature.
Calculation with units of expression	**Sugar % as follows** $$S = \frac{100\,(P - I)}{132.56 - 0.0794\,(13-m) - 0.53\,(t - 20)}$$ Where, P = direct reading, normal solution I = Invert reading, normal solution

	t = Temperature at which readings are made m = g of total solids from original sample in 100 mL inverted solution (solid by refractometer multiplied by specific gravity of solution) For Bura Invert the solution with HCl. Conduct the titration and calculate as given under Total sugars % expressed as sucrose = Total reducing sugars % x 0.95
Inference **(Qualitative Analysis)**	NA
Reference	A.O.A.C 21st edn, Official Method of Analysis (2019) method no. 925.48 Sucrose in Sugars and Syrup.
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.042:2024	**Revision No. & Date**	0.0
Scope	Icing sugar		
Caution	Concentrated Hydrochloric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Sucrose is inverted using acid and neutralized solution is titrated against Fehling solution. Subtraction of the reducing sugars provides the sucrose content.		
Apparatus/Instrument	General Apparatus and Glassware 1. Weighing balance 2. Conical Flask- 250 mL 3. 3Beakers- 250 mL 4. Volumetric Flask-100 mL 5. Volumetric Flask-250 mL 6. Volumetric Flask-500 mL 7. Boiling Water Bath 8. Pipettes, capacities 1 mL, 15 mL and 50 mL 9. Bunsen Burner, Tripod and Wire Gauze 10. Filter Paper 11. Burettes, capacity 50 mL,		
Materials and Reagents	1. Icing Sugar. 2. Distilled water. 3. Fehling's Solution (Soxhlet Modification). 4. Methylene Blue indicator. 5. Concentrated Hydrochoric Acid. 6. Asbestoses. 7. Disodium Hydrogen Phosphate, $Na_2HPO_4.12H_20$. 8. Glacial Acetic Acid, M20= 1.05 g/mL. 9. Acetic Acid Solution, approximately 5 mol/L. 10. Potassium Sodium Tartrate (Rochelle or Seignette Salt) 11. Copper sulphate Pentahydrate, $CuSO_4.5H_20$. 12. Sodium carbonate, anhydrous. 13. Soluble Starch 14. Hydrochloric acid, approximately 1 mol/L. 15. Hydrochloric acid, approximately 2 mol/L. 16. Ofner solution, Modified. 17. Potassium Iodate Solution, 0.01667 mol/L— 18. Starch Solution Indicator 19. Potassium Iodide, KI 20. Sodium Thiosulphate Solution, 0.0333 mol/L .		

	21. Iodine Solution, 0.01667 mol/L.
Preparation of Reagents	1. Fehling's Solution (Soxhlet Modification) — Prepared by mixing immediately before use, equal volumes of Solution A and Solution B. 2. Solution A — Dissolve 34.679 g of copper sulphate (CuSO4. 5H20) in water, add 0.5 mL of concentrated sulphuric acid of sp gr 1.84 and dilute to 500 mL in a volumetric flask. Filter the solution through prepared asbestos. 3. Solution B— Dissolve 173 g of Rochelle salt (potassium sodium titrate, $KNaC_4H_4O_6$,4H20)) and 50 g of sodium hydroxide, in water, dilute to 500 mL in a volumetric flask and allow the solution to stand for two days. Filter this solution through prepared asbestos. 4. Ofner Solution, Modified - Weigh out 7.0 g copper sulphate pentahydrate), 10.0 g sodium carbonate 300 g potassium sodium tartrate and 50 g disodium hydrogen phosphate in a 1000 mL flask. Dissolve in approximately 900 mL water (heat slightly to dissolve if necessary). Heat the solution for 2 h in a boiling water bath. Cool down to room temperature and fill up to the mark. Add approximately 10 g activated carbon and stir for 5-10 min. Filter the solution. 5. Potassium Iodate Solution, 0.01667 mol/L -Weigh out 3.5667 g potassium iodate KIO_3. Transfer to a 1000 mL volumetric flask, dissolve in water and fill to the mark 6. Starch Solution Indicator for Iodine — Dissolve 1 g of soluble starch in 100 mL saturated sodium chloride solution. Bring the solution to the boil for a few min. 7. Sodium Thiosulphate Solution, 0.0333 mol/L — Dilute a 0.1 mol/L sodium thiosulphate solution three fold with water and standardize with potassium iodate. Dissolve 2 g of potassium iodide in 10 mL water. Add 5 mL of approximately 2 mol/L hydrochloric acid and 10.0 mL of 0.01667 mol/L potassium iodate solution. Cover the flask with a watch glass, shake gently and leave the solution in the dark for approximately 30 min. Titrate the iodine formed with the sodium thiosulphate solution to complete decolorization, adding 1 mL of Starch indicator immediately before the endpoint. Calculate the factor f_{TH} of the thiosulphate solution: $$f_{TH} = \frac{30.96}{V_{TH}}$$ where V_{TH} = mL of sodium thiosulphate solution titrated. NOTE — f_{TH} corrects the used iodine solution to the experimentally determined value of 0.016 15 mol/1, for which 1 ml corresponds to 1 mg reducing sugars.

	8. Iodine Solution, 0.01667 mol/L- Dilute a 0.05 nlol/1 iodine solution three-fold with water and standardize with the 0.0333 mol/L sodium thiosulphate solution). Pipette 25.0 mL of the iodine solution into a 300 mL Erlenmeyer flask. Add 5 mL of 5 mol/1acetic acid and, after gently shaking the mixture, titrate back with the 0.0333 mol/L sodium thiosulphate solution Add I mL of starch indicator just before the endpoint is reached. Calculate the factor f_1 of the iodine solution: ‘ $f_1 = \frac{f_{Th} \, x V_{Th}}{2\,5}$ Where V_{TH} V_{TH}= mL of sodium thiosulphate solution titrated, and f_{TH} = Correction factor for the Sodium thiosulphate solution. 9. Methylene Blue indicator — Dissolve 0.2 of methylene blue in water and dilute to 100 mL. 10. Concentrated Hydrochloric acid — sp gr 1.16.
Sample Preparation	Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container.
Method of analysis	1. Weigh accurately about 5 g of the sample in a watch glass. 2. Transfer this quantity into a beaker, add about 50 mL of distilled water and warm the mixture in a water bath at 50 to 60 °C for about 5 min to dissolve the sucrose content of the sample. 3. Cool and filter through a Whatman filter paper No. 40 or equivalent. Collect the filtrate carefully in a 100 mL volumetric flask. 4. Wash the beaker and the insoluble residue of starch in the filter paper, carefully with water. 5. Make up the volume of the filtrate to 100 mL. **(B)Inversion** **1.** Take 10 mL of this solution in a conical flask and add 1.5 mL of concentrated hydrochloric acid and about 10 mL of water. **2.** Heat the flask at 60 to 70 °C for 10 min in a water bath. **3.** Cool immediately and transfer quantitatively the inverted solution to a volumetric flask and make up the volume to 100 mL **4.** Estimate reducing sugars in the inverted solution. **5.** Table 1: Invert Sugar Factors for 25 ml of Fehling’s Solution **HOT VALUE** **1.** Mix 50 mL of the prepared solution with 50 mL of the Ofner solution). 2. Add some pumice pieces to the mixture. 3. Bring the mixture to the boil within 4 to 5 min using the Bunsen burner, the tripod and the wire gauze. Boil for exactly 5 min. 4. Note: the start of boiling is once numerous steam bubbles break over the whole surface.

	5. Cool the mixture down in a water bath with cold running water. 6. After approximately 10 min the mixture should have reached room temperature. 7. Add 1 mL concentrated acetic acid. Add iodine solution until the colour of the mixture turns a typical iodine colour. 8. This procedure dissolves the formed Cu_2O with an excess of iodine. 9. The surplus iodine should be so high that between 10 mL and 15 mL of sodium thiosulphate) are consumed on back titration. 10. Add 15 mL of the 1mol/L Hydrochloric acid by pouring it down the inner side of the flask so that the residual droplets are washed down into the solution. 11. Cover the flask with a watch glass and move it gently for 2 min until the precipitate of Cu_2O is completely dissolved. 12. Titrate the sample with 0.0333 mol/L sodium thiosulphate Add 1 mL of starch solution immediately before the endpoint is reached. 13. Repeat the above procedure with another prepared solution mixed with Ofner solution and record the average of the two replicated V_1, and V_2 for iodine and thiosulphate respectively. **Cold Value** **1.** Mix 50 mL of the prepared sample with 50 mL of the Ofner solution. **2.** Leave the mixture at room temperature for 10 min. Repeat the procedure outlined in hot value Record values V_3 and V_4. **BLANK VALUE** **1.** Mix 50 mL of water with 50 mL of the Ofner solution. Repeat the procedure outlined in Hot value. Record the values V_5 and V_6
Calculation with units of expression	Expression of Results Added amount of iodine for hot value = V_1 Added amount of thiosulphate for hot value = V_2 Added amount of iodine for cold value = V_3 Added amount of thiosulphate for cold value = V_4 Added amount of iodine for blank value = V_5 Added amount of thiosulphate for blank value = V_6 Corrected consumption of 0.01667 mol/L iodine Solution: Calculated hot value,A = $(V_1 \times f_1,) - (V_2 \times f_{Th})$ Calculated cold value, B = $(V_3 \times f_1,) - (V_4 \times f_{Th})$ Calculated blank value,C = $(V_5 \times f_1,) - (V_6 \times f_{Th})$ Where f_1 = is the factor of the iodine solution Sucrose correction, D, is 0.1 mL sucrose in the reaction mixture.

1) Invert sugar, mg/Kg = $\frac{(A- B- C- D) X1OOO}{S}$ s

where S = the amount of sample in 50 mL of prepared solution .

2) Sucrose, percent = $\frac{0.95\ (Q-W-R)}{w}$

Where

W= Value in col 3 of table 1.

Q =mass, in g, of the material taken for the test.

R = percent reducing sugars by mass

Table 1

Invert Sugar Factor for 25 ml of Fehling's Solution

Titre	Invert Sugar Factor	Reducing Sugar (as Content Anhydrous Dextrose per 100 ml of Solution)
(1)	(2)	(3)
15	120.2	80 I
16	120.2	751
17	120.2	707
18	120.2	668
19	120.3	638
20	120.3	601.5
21	120.3	572.9
22	120.4	547.3
23	120.4	523.6
24	120.5	501.9
25	120.5	482.0
26	120.6	463,7
27	120.6	446.8
28	120.7	431.1
29	120.7	316.4
30	120.8	402.7
31	120.8	389.7
32	120.8	377.6
33	120.9	366.3
34	120.9	355.6
35	121.0	345.6
36	121.0	336.3
37	121.1	327.4
38	121.2	318.8
39	121.2	310.7

		40	121.2	303.1	
		41	121.3	295.9	
		42	121.4	289.0	
		43	121.4	282.4	
		44	121.5	276.1	
		45	121.6	270.1	
		46	121.6	264.3	
		47	121.6	258.8	
		48	121.7	253.5	
		49	121.7	248.4	
		50	121.8	243.6	
Inference (Qualitative Analysis)	NA				
Reference	IS : 15279 - 2003 Sugar and Sugar Products				
Approved by	Scientific Panel on Methods of Sampling and Analysis				

एफएसएसएआई fssai भारतीय खाद्य सुरक्षा और मानक प्राधिकरण Food Safety and Standards Authority of India स्वास्थ्य और परिवार कल्याण मंत्रालय Ministry of Health and Family Welfare	**Determination of Reducing Sugars in Cane sugar and refined sugar, Gur or Jaggery and Cane Gur/Cane Jaggery: Method A**		
Method No.	FSSAI 04C.043:2024	**Revision No. & Date**	0.0
Scope	Cane sugar and refined sugar, Gur or Jaggery and Cane Gur/Cane Jaggery		
Caution	Wear mask and gloves during analysis		
Principle	Invert sugar reduces the copper in Fehling's solution to red, insoluble cuprous oxide. The sugar content in a food sample is estimated by determining the volume of the unknown sugar solution required to completely reduce a known volume of Fehling's solution. Glucose and other sugars are capable of reducing oxidizing agents and are called reducing sugars		
Apparatus/Instrument	General Apparatus and Glassware 1. Volumetric flasks 2. Burette 3. Pipettes 4. Conical flasks 5. Bunsen burner 6. Wire guage 7. Weighing balance		
Materials and Reagents	1. Copper sulphate ($CuSO_4.5H_2O$) 2. Rochelle salt (Potassium Sodium tartrate) (K Na $C_4H_4O_6$. $4H_2O$) 3. Sodium hydroxide 4. Zinc acetate. 5. Acetic acid. 6. Potassium ferrocyanide 7. Methylene blue		
Preparation of Reagents	(1) Fehling A: Dissolve 69.28 g copper sulphate in distilled water. Dilute to 1000 mL. Filter and store in amber coloured bottle. (2) Fehling B: Dissolve 346 g Rochelle salt and 100 g NaOH in distilled water. Dilute to 1000 mL. Filter and store in amber coloured bottle. (3) Carrez 1 – Add 21.9 g Zinc acetate and 3 mL acetic acid in a 100 mL volumetric flask. Make up the volume with water. (4) Carrez 2 – 10.6%, w/v aqueous solution of Potassium ferrocyanide. (5) Methylene Blue Indicator: Prepare 1%, w/v of methylene blue solution in distilled water.		
Sample Preparation	Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container.		
Method of analysis	1. Weigh accurately about 5 g sample, transfer to a 200 mL volumetric flask, dissolve in warm water& dilute to about 150 mL. 2. In case solution is not clear, add 5 mL of Carrez 1 solution followed by 5 mL of Carrez 2 solution.		

	3. Make up to 200 mL. Filter through a dry filter paper. 4. Titrate the solution obtained as such to determine % Reducing sugars. 5. Preliminary Titration: Pipet 5 mL each of Fehling A and B into 250 mL conical flask. Mix and add about 10 mL water and a few boiling chips or glass beads. 6. Dispense sample solution from burette. Heat the flask to boiling. 7. Add 3 drops of methylene blue indicator. 8. Continue the addition of solution dropwise until the blue colour disappears to a brick-red end point. (The concentration of the sample solution should be such that the titre value is between 15 and 50 mL). 9. Note down the titre value. 10. Final Titration: Pipet 5 mL each of Fehling A and B. Add sample solution about 2 mL less than titre value of the preliminary titration. Heat the flask to boiling within 3 min and complete the titration. Perform the titration in duplicate and take the average.
Calculation with units of expression	Calculate the reducing sugar % as shown below. $\text{Reducing Sugars \%} = \frac{\text{Dilution x Factor of Fehling (in g) X 100}}{\text{Weight of sample x Titre value}}$
Inference (Qualitative Analysis)	NA
Reference	1. Luff school method, GS 1-5 for reducing sugars, 2. Modified offner method, GS 2-6 for white ad refined sugars 3. IS 15729 also has adopted the modified Offner Method
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.044:2024	**Revision No. & Date**	0.0
Scope	Cane sugar and refined sugar, Gur or Jaggery and Cane Gur/Cane Jaggery		
Caution	Concentrated Hydrochloric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	The method measures the reducing power of solutions of white sugar containing reducing substances, for example, invert sugar in a weak alkaline solution of a Cu++ complex with tartrate. The complex formed between Cu++ ions and potassium sodium tartrate is reduced by reducing sugars to univalent Cu+ which is precipitated as Cu2O. The precipitated Cu2O is then determined by iodometric titration. The Cu2O is oxidized by an excess is back titrated with sodium thiosulphate.		
Apparatus/Instrument	General Apparatus and Glassware 1. Analytical Balance, capable of weighing to the nearest 0.1 mg. 2. Precision Balance, capable of weighing to the nearest 0.1 g. 3. Burettes, capacity 50 ml, 4. Erlenmeyer Flasks, capacity 300 ml. 5. Volumetric Flasks, 1000 ml and 200 ml. 6. Pipettes, capacities 1 ml, 15 ml and 50 ml. 7. Watch Glasses, to cover Erlenmeyer flasks. 8. Bunsen Burner, Tripod and Wire Gauze/Hot plate 9. Boiling Water Bath 10. Water Bath with Cold Running Water 11. Filter Paper		
Materials and Reagents	1. Activated Carbon, powdered. 2. Small Pumice Pieces 3. Disodium Hydrogen Phosphate 4. Glacial Acetic Acid, $\rho = 1.05$ g/ml. 5. Acetic Acid Solution, approximately 5 mol/l. 6. Potassium Sodium Tartrate (Rochelle Salt) 7. Copper Sulphate Pentahydrate, 8. Sodium Carbonate, anhydrous. 9. Hydrochloric Acid, approximately 1 mol/1. 10. Hydrochloric Acid, approximately 2 mol/l. 11. Potassium Iodide 12. Soluble Starch 13. Potassium Iodate 14. Sodium Thiosulphate 15. Iodine		
Preparation of Reagents	(1) (***Ofner Solution, modified:*** Weigh out 7.0 g copper *sulphate* pentahydrate, 10.0 g sodium carbonate, 300 g potassium sodium		

tartrate and 50 g disodium hydrogen phosphate in a 1000 ml flask. Dissolve in approximately 900 ml water (heating slightly to dissolve if necessary). Heat the solution for 2 h in a boiling water bath. Cool down to room temperature and fill up to the mark. Add approximately 10 g activated carbon and stir for 5-10 min. Filter the solution.

(2) **Starch Solution:** Dissolve 1 g of soluble starch in 100 ml saturated sodium chloride solution. Bring the solution to boil for a few minutes.

(3) ***Potassium Iodate Solution, 0.01667*** mol/l: Weigh out 3.5667 g potassium iodate, previously dried for 3h at 100 ^{0}C. Transfer to a 1000 ml volumetric flask, dissolve in water and fill to the mark.

(4) **Sodium thiosulphate solution, 0.0333 mol/l:** Prepare 0.1 ml/l sodium thiosulphate solution by weighing out 24.818 g of Sodium thiosulphate *pentahydrate* and dissolving it in 400 ml distilled water in 1000 ml volumetric flask. Make up to the mark with water. Dilute this solution three-fold with water.

Standardization and determination of factor for thiosulphate solution f_{Th} *:* Dissolve 2 g of potassium iodide in 10 ml water into a 250 ml Erlenmeyer flask. Add 5 ml of approximately 2 mol/l hydrochloric acid and 10.0 ml of 0.01667 mol/1 potassium iodate solution. Cover the flask with a watch glass, shake gently and leave the solution in the dark for approximately 30 min. Titrate the iodine formed with the sodium thiosulphate solution to complete decolonization, adding 1 ml of starch indicator immediately before the endpoint. Calculate factor for thiosulphate solution f_{Th}**:**

$$f_{Th} = 30.96/V_{Th}$$

Where:

V_{Th} = ml of sodium thiosulphate solution titrated.

(5) **Iodine solution 0.01667 mol/l:** Prepare 0.05 ml/l iodine solution by weighing out 53 g of potassium iodide and dissolving it in 50 ml distilled water in 1000 ml volumetric flask. Transfer 12.690 g iodine into this flask, dissolve and make up to the mark with water. Dilute this solution three-fold with water.

Standardization and determination of factor for iodine solution f_I *:* Pipette 25.0 ml of the iodine solution into a 250 ml Erlenmeyer flask. Add 5 ml of 5 mol/1acetic acid and, after gently shaking the mixture, titrate back with the 0.0333 mol/1sodium thiosulphate solution. Add 1 ml of starch indicator just before the endpoints is reached. Calculate factor for iodine solution f_I**:**

$$f_I = V_{th} \text{ X } f_{Th}/25$$

Where:

V_{Th} = ml of sodium thiosulphate solution titrated.

f_{Th} = Correction factor for the sodium thiosulphate solution

Sample Preparation	Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container.
Method of analysis	Weigh 40 g of sugar and dissolve it in distilled water in 200 ml volumetric flask. Make up to the mark with distilled water. 1. **Determine added amount of iodine (V_1) and thiosulphate (V_2) for Hot Value:** Mix 50.0 ml of the above prepared solution with 50.0 ml of the Ofner solution in 250 ml Erlenmeyer flask. Add some pumice pieces to the mixture. Bring the mixture to the boil within 4 to 5 min using hot plate. Boil for exactly 5 min. Note the start of boiling is once numerous steam bubbles break over the whole surface. Cool the mixture down in a water bath with cold running water. After approximately 10min the mixture should have reached room temperature. Add 1 ml glacial acetic acid followed by iodine solution until the colour of the mixture turns a typical iodine colour. Then, add 15 ml of the 1mol/l hydrochloric acid by pouring it down the inner side of the flask so that the residual droplets are washed down into the solution. Cover the flask with a watch glass and move it gently for 2 min until the precipitate (Cu2O) is completely dissolved. Titrate the sample with 0.0333 mol/1 sodium thiosulphate. Add 1 ml of starch solution immediately before the endpoint is reached. Repeat the above procedure with another prepared solution mixed with Ofner solution and record the average of the two replicated V_1, and V_2 for iodine and thiosulphate respectively. 2. **Determine added amount of iodine (V_3) and thiosulphate (V_4) for Cold Value:** Mix 50.0 ml of the above prepared solution with 50.0 ml of the Ofner solution in 250 ml Erlenmeyer flask. Leave the mixture at room temperature for 10 min. Add 1 ml glacial acetic acid followed by iodine solution until the colour of the mixture turns a typical iodine colour. Then, add 15 ml of the 1mol/l hydrochloric acid by pouring it down the inner side of the flask so that the residual droplets are washed down into the solution. Cover the flask with a watch glass and move it gently for 2 min until the precipitate (Cu2O) is completely dissolved. Titrate the sample with 0.0333 mol/1 sodium thiosulphate. Add 1 ml of starch solution immediately before the endpoint is reached. Repeat the above procedure with another prepared solution mixed with Ofner solution and record the average of the two replicated V_3, and V_4 for iodine and thiosulphate respectively. 3. **Determine added amount of iodine (V_5) and thiosulphate (V_6) for Blank Value:** Mix 50.0 ml of distilled water instead of prepared solution with 50.0 ml of the Offner solution in 250 ml Erlenmeyer flask. Leave the mixture at room temperature for 10 min. Repeat the procedure outlined for cold

	value. Record the average of the two replicated V_5, and V_6 for iodine and thiosulphate respectively.
Calculation with units of expression	Calculate the reducing sugar % as shown below. $\text{Reducing Sugars \%} = \frac{(A-B-C-1)}{100}$ Where: $A = (V_1 \times f_I) - (V_2 \times f_{Th})$ $B = (V_3 \times f_I) - (V_4 \times f_{Th})$
Inference (Qualitative Analysis)	NA
Reference	1. Modified ofner method, ICUMSA GS 2-6 for white ad refined sugars 2. IS : 15279 -2003 SUGAR AND SUGAR PRODUCTS
Approved by	Scientific Panel on Methods of Sampling and Analysis

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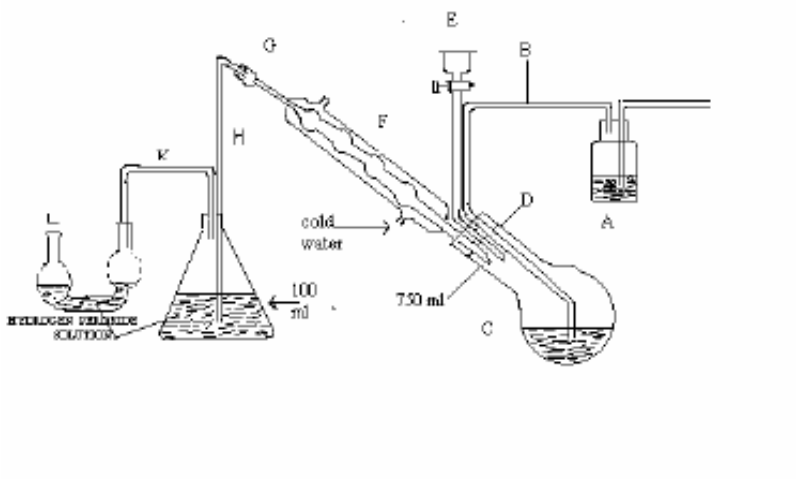
Method No.	FSSAI 04C.045:2024	**Revision No. & Date**	0.0
Scope	White sugar and Refined sugar, Misri		
Caution	Concentrated Hydrochloric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Sulphur dioxide is bubbled out from the sample solution using heating and passing carbon dioxide gas into in a flask. Dissolved Sulphur dioxide is estimated using alkali and indicator.		
Apparatus/Instrument	General Apparatus and Glassware 1. The apparatus as assembled is shown below:  **Assembly for determination of Sulphur dioxide**		
Materials and Reagents	1. Sodium Carbonate. 2. Bromo phenol Blue. 3. Hydrogen peroxide solution (30%). 4. Concentrated Hydrochloric acid- sp.gr. 1.16 5. Carbon dioxide gas- from a cylinder. 6. Sodium hydroxide. 7. Ethyl alcohol. 8. Barium hydroxide. 9. Potassium permanganate.		
Preparation of Reagents	1. Sodium Carbonate Solution [10 percent (m/v)]- Sodium carbonate (10 g) dissolved in water (100 mL).		

	2. Bromo phenol Blue Indicator Solution – Dissolve 0.1 g of bromo phenol blue in 3.0 mL of 0.05 N sodium hydroxide solution and 5mL of ethyl alcohol (90 %, v/v) by gently warming. Make up the volume of the solution with ethyl alcohol (20 %, v/v) to 250mL in a volumetric flask. 3. Hydrogen peroxide solution – Dilute 30 percent (m/v) hydrogen peroxide solution with about twice its volume of water and neutralize the free sulphuric acid that may be present in the hydrogen peroxide solution with barium hydroxide solution, using bromo phenol blue indicator solution. Allow the precipitate of barium sulphate to settle and filter. Determine the concentration of hydrogen peroxide in the filtrate by titrating with standard potassium permanganate solution. Dilute the filtrate with cold water so as to obtain a 3 percent (m/v) solution of hydrogen peroxide. 4. Standard sodium hydroxide solution - approximately 0.1 N, standardized at the time of the experiment using bromo phenol blue indicator solution.
Sample Preparation	**Sugar boiled confectionery & Lozenges** If composition of entire product is desired, grind and mix thoroughly. If product is composed of layers or of distinctly different portions and it is desired to examine these individually, separate with knife or other mechanical means as completely as possible, and grind and mix each test portion thoroughly. **Cane sugar and refined sugar** Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container.
Method of analysis	1. Assemble the apparatus as shown above. Introduce into the flask C, 300 mL of water and 20 mL of concentrated hydrochloric acid through the dropping funnel E. 2. Run a steady current of cold water through the condenser F. 3. Boil the mixture contained in the flask for a short time to expel the air from the system in current of carbon dioxide gas previously passed through the wash bottle A.

	4. Weigh accurately about 100 g of the sample and mix with the minimum quantity of water so as to make the diluted sample easily flow down to the dropping funnel. 5. Introduce the diluted material into the flask C through the dropping funnel E. Wash the dropping funnel with a small quantity of water and run the washing into the flask C. 6. Again boil the mixture contained in the flask C in a slow current of carbon dioxide gas (passed previously through the wash bottle A) for one hour. 7. Just before the end of the distillation, stop the flow of water in the condenser. (This causes the condenser to become hot and drives over residual traces of sulphur dioxide retained in the condenser.) When the delivery tube H, just above the Erlenmeyer flask j, becomes hot to touch, remove the stopper J immediately. 8. Wash the delivery tube H and the contents of the Peligot tube L with water into Erlenmeyer flask. 9. Cool the contents of the Erlenmeyer flask to room temperature, add a few drops of bromo phenol blue indicator 10. Titrate with standard sodium hydroxide solution.(Bromo phenol blue is unaffected by carbon dioxide and gives a distinct change of color in cold hydrogen peroxide solution). 11. Carry out a blank determination using 20 mL of conc. hydrochloric acid diluted with 300 mL of water. **Note:** Rosaniline method can also be used as alternative method for determination of sulphur dioxide.
Calculation with units of expression	Sulphur dioxide, mg/kg = $\frac{0.032000 \quad (V-v) \times 1000 \times 1000 \times N}{W}$ Where, V = volume in mL of standard sodium hydroxide solution required for the test with the material v = volume in mL of standard sodium hydroxide solution required for the blank determination; N = normality of standard sodium hydroxide solution; and

	W = weight in g of the material taken for the test.
Inference (Qualitative Analysis)	NA
Reference	I.S.I Handbook of Food Analysis (Part II) -1984
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.046:2024	**Revision No. & Date**	0.0
Scope	White sugar and Refined sugar, Misri		
Caution	Concentrated Hydrochloric acid is corrosive, causes burns. Wear mask and gloves during analysis.		
Principle	The sugar is dissolved and reacted with formaldehyde and a rosaniline solution. The colour of the sulphite/rosaniline complex is measured spectrophotometrically at a wavelength of 560 nm and compared to a standard graph.		
Apparatus/Instrument	General Apparatus and Glassware 1. Spectrophotometer or Calorimeter, for use at approximately 560 nm. 2. Volumetric Flasks, (100, 500 and 1000 mL). 3. Graduated Pipette, 10 mL. 4. Pipettes, 2, 10 and 25 mL. 5. Burette, 10 mL, graduated by 0.05 mL 6. Test Tubes 7. Analytical Balance, capable of weighing to the nearest 0.1 mg.		
Materials and Reagents	1 Rosaniline Hydrochloric Solution 2 Formaldehyde Solution 3 Pure Sucrose Solution 4 Sodium Hydroxide Solution, 5 Iodine Solution 6 Concentrated Hydrochloric Acid, 7 Iodine (Starch) Indicator, 8 Sodium Thiosulphate Solution		
Preparation of Reagents	1. Rosaniline Hydrochloric Solution (Saturated) — Suspend 1 g of rosaniline hydrochloride in 100 mL of distilled water, heat to 50 °C and cool with shaking. After standing for 48 h, filter the solution. 2. Decolourized Rosaniline Solution — Transfer 4 mL of saturated rosaniline hydrochloride solution to a 100 mL volumetric flask. After addition of concentrated hydrochloric acid (6 mL) make the mixture up to the mark. Decolourization takes places in short time		

	3. Formaldehyde Solution (approximately 0.2 gt 100 mL) — Dilute 5 mL of analytical reagent grade formaldehyde solution, p20= 1.070– 1.080 to 1000 mL. 4. Pure Sucrose Solution — Dissolve 100 g of analytical reagent grade sulphite-free sucrose in water and make up 1000 mL 5. Sodium Hydroxide Solution, 0.1 mol/L 6. Iodine Solution, 0.05 mol/L — Dissolve 20 g of analytical reagent grade iodate-free potassium iodide in 40 mL of distilled water in a 1000 mL volumetric flask. After the addition of 12.69 g of analytical reagent grade iodine shake the flask until all the iodine is dissolved and then make up to the mark with distilled water. 7. Hydrochloric Acid Solution, approximately 1 mol/L. 8. Iodine (Starch) Indicator, ready-made, or a starch solution. 9. Sodium Thiosulphate Solution, 0.1 mol/L— Dissolve 24.817 g of analytical reagent grade sodium thiosulphate pentahydrate in 200 mL of distilled water in a 1000 mL volumetric flask and then makeup to the mark. 10. Standard Sulphite Solution — Dissolve approximately 2.5 g of general purpose reagent grade sodium sulphite heptahydrate in sucrose solution and make up to 500 mL with this pure sucrose solution. Determine the titre of this solution as follows. Place 25 mL of the 0.05 mol/L iodine solution in a 300 mL conical flask and add 10 mL of the 1 mol/L hydrochloric acid solution followed by approximately 100 mL of distilled water. Pipette 25 mL of standard sulphite solution into this flask while swirling the flask. Then titrate the excess iodine with the 0.1 mol/L sodium thiosulphate solution until the contents of the flask are pale straw colour. Then add the iodine (starch) indicator to the flask and continue the titration until the blue colour disappears. Record the titre, t. 11. Dilute Standard Sulphite Solution — Dilute 5 mL of standard sulphite solution to exactly 100 mL with pure sucrose solution. The exact value of the sulphite content, c, is calculated as follows from the titre, t. 12. c = (25 – t) x 3.203 x 2 mg SO_2/mL NOTE— Users of this method are advised to consult their national health and safety legislation and chemical suppliers before handling rosaniline hydrochloride, formaldehyde and the

	other reagents here mentioned.
Sample Preparation	Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container.
Method of analysis	A) C**olour Development** 1. Dissolve 10-40 g of a sample of white sugar in distilled water in a 100 mL volumetric flask 2. After addition of 0.1 mol/L sodium hydroxide solution (4 mL) make the contents of the flask up to the mark and mix: 3. For levels 0-5 mg SO_2/kg use 40 g of sample, 5-15 mg SO_2/kg use 20 g of sample, 15-30 mg SO_2/kg use 10 g of sample 4. Transfer a 10 mL aliquot to a clean, dry test tube. 5. Add 2 mL of decolorized rosaniline solution and 2 mL of formaldehyde solution and allow the tube to stand at room temperature for 30 min. 6. Measure the absorbance in a 1 cm cell in a spectrophotometer at about 560 nm using distilled water as a reference. B) **Standard Curve** 1. Pipette aliquots of the dilute standard sulphite solution (1, 2, 3, 4, 5 and 6 mL) into a series of 100 mL volumetric flasks. 2. Take an empty flask as well for the zero sulphite level 3. To each flask add 4 mL of 0.1 mol/L sodium hydroxide and make the contents up to the mark with pure sucrose solution) and mix. 4. From each flask transfer a 10 mL aliquot to a clean, dry test tube 5. Add 2 mL of decolorized rosaniline solution and 2 mL of formaldehyde solution and allow the tubes to stand at room temperature for 30 min. 6. Measure the absorbance and plot the results on a graph. 7. The amount of SO_2in each test tube is: $\frac{c \times n}{10}$ µg SO_2. where n = the number of mL of dilute sulphite added to each 100 mL flask
Calculation with units of expression	Sulphur dioxide, mg/kg = $\frac{(\mu g\ SO_2 \text{ from graph}) \times 10}{\text{Sample weight(g)}}$

Inference (Qualitative Analysis)	NA
Reference	IS 15279:2003 SUGAR AND SUGAR PRODUCTS
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.047:2024	**Revision No. & Date**	0.0
Scope	Sugar and Sugar Products		
Caution	Wear mask and gloves during analysis		
Principle	Specific conductivity is determined by measuring the conductivity of a solution kept in a cell and multiplying it with the cell constant.		
Apparatus/Instrument	General Apparatus and Glassware (Page 3 and 4) 1. Conductivity bridge with magic eye indication for measuring the conductivity directly.		
Materials and Reagents	1. Sodium / Potassium dichromate 2. Conductivity water – of specific conductivity not more than 3.0×10^{6}. 3. Potassium chloride. 4. Chloroplatinic acid. 5. Lead acetate 6. Concentrated Sulphuric acid		
Preparation of Reagents	1. Chromic acid solution. - Using rubber gloves, transfer 20 g of powdered sodium/potassium dichromate into 1Lone liter glass beaker. Add water in small amounts and mix well to form a paste, and stir thoroughly with a glass stirring rod. While stirring continuously, add 300 mL of concentrated sulphuric acid to the paste. Transfer the solution carefully into a glass bottle and stopper it. 2. Potassium Chloride Solution – 0.02 N, accurately prepared. 3. Chloroplatinic acid solution – Dissolve 3 g of Chloroplatinic acid and 0.02 – 0.03 g of lead acetate in 100 mL water.		
Sample Preparation	Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, after withdrawl of test portions for analytical determinations.		
Method of analysis	**Platinizing the electrodes of the conductivity cell** 1. Wash the electrodes of conductivity cell first with warm chromic acid solution and then several times with distilled water. 2. Support the electrodes in an inclined position in the chloroplatinic acid solution and connect by way of a commutator to a 4 volt lead accumulator and rheostat.		

	3. Adjust the current so that the evolution of gas is slow. 4. Reverse the current every 30 sec. 5. Thus continue to pass the current for 15 min. 6. Disconnect the conductivity cell wash it with distilled water thoroughly and fill with dil. solution of sulphuric acid. 7. Electrolyze the solution of sulphuric acid for ½ hour to remove occluded gases, reversing the current every 30 sec. 8. Wash the cell wall with conductivity water. **Note:** The cleanliness of the cell is confirmed by determining the conductivity of the conductivity water, washing out the cell and making a second determination of the conductivity water. Two successive determinations shall give concordant measurement of the conductivity, if the cell is clean. **Determination of the cell constant** 1. Wash the conductivity cell with conductivity water. 2. Then rinse with the standard Potassium chloride solution. 3. Transfer sufficient quantity of Potassium chloride solution so that the electrodes are well within the solution, taking care that no air bubbles are enclosed between the electrodes. 4. Place the conductivity cell in a thermostat. Maintain the temperature of the thermostat at 35 ± 1°C. 5. Ensure that all the connections made are with fairly thick copper wire and tight. 6. When Potassium chloride solution has attained the temperature of the bath, measure the observed conductivity of the solution. 7. Report twice the measurement by replacing a fresh Potassium Chloride solution. **Determination of specific conductivity** 1. Dissolve 10 g of the sample (accurately weighed) in 200 mL of conductivity water. 2. Wash the conductivity cell thoroughly with distilled water and then with conductivity water and later rinse with test solution twice. 3. Determine the observed conductivity at 35 °C. Repeat with a fresh

	sample of test solution and take the average value. 4. Determine the conductivity of conductivity water at 35 °C in the same manner.
Calculation with units of expression	Calculate the cell constant as follows $K = \frac{C}{O_1}$ Where, K = cell constant C = specific conductivity of potassium chloride solution at 35°C, that is 3.3.1X 10^3 Mhos / cm O_1 = Observed conductivity of potassium chloride solution Calculate the specific conductivity x 10^6 of 5 % (w/ v) aqueous solution at 35°C as follows: $S = [O_2 – (0.9 X O_3)] x K x 10^6$ Where, S = specific conductivity of test solution X 10^6 O_2 = observed conductivity of test solution O_3 = observed conductivity of conductivity water K = cell constant
Inference (Qualitative Analysis)	NA
Reference	I.S.I. Handbook of food analysis (Part II) – 1984, page 7.
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Method No.	FSSAI 04C.048:2024	**Revision No. & Date**	0.0
Scope	Cane sugar and refined sugar, Khandsari Sugar		
Caution	Wear mask and gloves during analysis		
Principle	The specific conductivity of a white sugar solution at a concentration of 28g / 100g (28 %, w/v) is determined. The equivalent ash is calculated by the application of a conventional factor.		
Apparatus/Instrument	General Apparatus and Glassware 1. Conductivity bridge with magic eye indication for measuring the conductivity directly. 2. Volumetric Flasks, 100,500 and I000 ml 3. Pipettes, 10mL, conforming to Class A of IS 1117. 4. Analytical Balance, capable of weighing to the nearest 0.1 mg.		
Materials and Reagents	1. Purified Water 2. Potassium Chloride		
Preparation of Reagents	1. Purified Water — for preparation of all solutions (sugar and potassium chloride) use twice-distilled or deionized water with a conductivity of less than 2 µS/cm. 2. 2 Potassium Chloride, 0.01 mol — Weigh out 745.5 mg after first dehydrating by heating to 500 °C (dull red heat). Dissolve in water in a 1 L volumetric flask and make up to the mark. 3. Potassium Chloride, 0.0002 mol/L — Dilute 10 mL of potassium chloride solution, 0.01 mol/L and make up to the mark in a 500 mL volumetric flask. This solution has a conductivity of 26.6 + 0.3 µS/cm at 20 °C (after deduction of the specific conductivity of the water used).		
Sample Preparation	Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, after withdrawal of test portions for analytical determinations.		
Method of analysis	1. Dissolve 31.3 g ± 0.1 g of sugar in water in a 100 mL volumetric flask and make up to volume at 20 °C (or dissolve 28.0 + 0.1 g of sugar in water to give a solution of mass 100.0 g). Wash the electrodes of conductivity cell first with warm chromic acid solution and then several times with distilled water.		

	2. In the case of liquids, the amount taken must be such that the test solution contains 31.3 g of solids/100 mL, or 28.0 g solids/100 g of solution. 3. After thorough mixing, transfer the solution into the measuring cell and measure the conductivity at 20+ 0.2 ^{O}C. Check the measurement.
Calculation with units of expression	If C, is the measured conductivity in µS/cm at 20 °C and if C_2 is the specific conductivity of the water at 20 °C, then the corrected conductivity (C_{28}) of the 28 g/100 g solution $C_{28} = c_1 - 0.35\ c_2$ and Conductivity ash, % $=6 \times 10^{-4} \times C_{28}$ **Temperature Correction** If the determination cannot be made at the standard temperature of 20 °C make a temperature correction to the final result provided that the range of* 5 °C is not exceeded. The correction is: $C_{20\,c} = \frac{C_T}{1+0.026(T-20)}$ where C_T = conductivity at temperature T°C NOTE -- The conductivity of the potassium chloride standard solution is given for a temperature of 20 °C. If the measurement cannot be made at the standard temperature of 20 °C. Then the conductivity of the potassium chloride standard solution has to be determined by the formula Conductivity of KCI at T °C = 26.6 [1+0.021 (T- 20)] in the range 20 + 5 °C.
Inference (Qualitative Analysis)	NA
Reference	IS 15279:2003 SUGAR AND SUGAR PRODUCTS
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Method No.	FSSAI 04C.049:2024	**Revision No. & Date**	0.0
Scope	White Sugar, Refined Sugar, Khandsari sugar, Bura Sugar, Icing Sugar, Misri, Gur Or Jaggery, Cane Jaggery, cane gur		
Caution	Wear gloves during analysis		
Principle	Sample is dissolved in hot water and insoluble matter is filtered and estimated.		
Apparatus/Instrument	General Apparatus and Glassware 1. Beaker 2. Filtration system. 3. Gooch Crucible with sintered glass filter (8.0mm) 4. Hot air oven. 5. Weighing balance		
Materials and Reagents	1. Distilled water 2. Water bath with heating system 3. Molisch reagent		
Sample Preparation	Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container.		
Method of analysis	1. Take 10 g of sample. 2. Add 200 mL hot distilled water and bring to boiling. 3. Allow to cool to room temperature. 4. Filter through a tared gooch crucible having a bed of asbestos or sintered glass filter. Wash the residue with hot water till the filtrate is sugar-free (perform Molisch test). 5. Dry the gooch crucible or sintered glass filter at 135 ± 2 °C and weigh. Express as % insoluble matter.		
Calculation with units of expression	$\% \text{ insoluble matter} = \frac{W1 - W}{M} \times 100$ M : Weight of the sample taken. W: Weight of the empty Gooch Crucible with sintered filter. W1: Weight of the Gooch Crucible with sintered filter and insoluble matter.		
Inference (Qualitative Analysis)	NA		

Reference	IS 12923:1990 Cane Gur(Jaggery)
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Method No.	FSSAI 04C.050:2024	**Revision No. & Date**	0.0
Scope	White Sugar, Refined Sugar, Bura Sugar, Icing Sugar, Misri, Gur Or Jaggery		
Caution	Wear gloves during analysis		
Principle	The sugar to be tested is dissolved in hot water and filtered through a membrane filter of pore size 8.0 mm. The membrane and the retained insoluble matter are thoroughly washed, dried and weighed. The insoluble matter content is calculated from the increase in mass of the membrane filter.		
Apparatus/Instrument	General Apparatus and Glassware 1. Membrane Filters, diameter about 50 mm, pore size 8.0 mm. 2. Glass Fibre Pre-Filters, with an acrylic binder for the modified procedure only. 3. Filtration Apparatus, comprising a holder for the membrane filter fitted into a conical filtration flask, of capacity 4 litre, connected with a vacuum system. 4. Stainless Steel Jug, capacity 2 litre with a stainless steel stirring rod. 5. Tweezers 6. Plastic Petri Dishes 7. Drying Oven, maintained between 60 and 65 ºC. 8. Square Mesh Sieve, diameter 20 cm, mesh size about 0.4 mm. Place the sieve in a level base pan containing hot distilled water, in such a way that the water is just in contact with the mesh of the sieve. Cover the sieve with a lid. 9. Analytical Balance, readable to 0.1 mg. 10. Balance, capacity 5 kg. Capable of weighing to the nearest 1 g.		
Materials and Reagents	1. Distilled water 2. Chromatographic Spray Reagent		
Preparation of Reagents	1. Chromatographic Spray Reagent.-1-naphthol/ phosphoric acid solution. Dissolve 1.0 g of 1-naphthol 100 ml of ethanol and add 10 ml of ortho-phosphoric and (P_{20}= 1.69 g/ml).		
Sample Preparation	Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container		
Method of analysis	Different procedures, 1 and 2, are used according to whether the sugar filters with a good rate or poorly. **Preparation of Membrane Filter** For white sugars with a good filtration rate: Wash the membranes by immersion in boiling distilled water for 6 min. Drain the excess water from the membrane and transfer individually to clean, dry petri		

dishes by using tweezers. Dry the membranes in their dishes with the lids removed for I h at 60 to 65°C in the drying oven. After drying replace the lids and cool for 30 min in a desiccator. Record the mass of the cooled membranes to the nearest 0.1 mg.

2) **Preparation of Membrane Filter and Pre-Filter**

For white sugar with poor filtration characteristics:

Wash the membranes by immersing them-in boiling distilled water for 6 min. Place a washed membrane into the filter holder and position a pre-filter on the top of the membrane. Pour 1500 ml of hot distilled water at about 95°C through the filter to remove water-soluble material from the pre-filter. Remove the membrane and the pre filter from the holder and place in a petridish. Dry the membrane and the pre-filter in the dish with the lid removed for 1.5 h at 60 to 65”C. After drying. Replace the lid and cool in a desiccator for 30 min. Weigh the membrane with the pre-filter to the nearest 0.1 mg.

Preparation of Sample Solution

1. For refined sugars with an expected insoluble matter content of 20 mg/kg or less, weigh 1000 ± 1 g of the sample directly into the stainless steel jug.

2. For plantation white sugars with an expected insoluble matter content 20 to 50 mg/kg reduce the sample mass to 500 ± 1 g.

3. For plantation white sugars with expected insoluble matter content greater than 50 mg/kg reduce the sample mass to 200± 1 g.

4. Add hot distilled water at about 95°C to the jug to give a final volume of about 1 800 ml.

5. Stir the mixture with the stainless steel rod and heat to about 95°C; continue stirring until all the sugar has dissolved.

NOTE –- Clothes for drying apparatus maybe a serious source of contamination. It is therefore important that all apparatus should be rinsed thoroughly with distilled water immediately prior to use, but not dried with a cloth.

Filtration of the Sugar Solution

1. Moisten a weighed membrane filter by floating it on distilled water in the petri dish.

2. Place the moistened filter in the fiber holder and pass the hot sugar

	solution through the membrane filter under reduced pressure. **3.** Carefully rinse the jug and stirring rod into the filter holder with hot distilled water. 4. Wash the retained insoluble matter and the membrane in the filter holder using a total volume of hot distilled wash water of about 1000 mL. NOTE —Do not allow air to be drawn through the membrane after washing, because there may be a significant amount of particulate matter in the atmosphere. In the case of white sugar with poor filtration characteristics moisten the membrane and pre-filter with distilled water and replace them in the filter holder ensuring that the pre-filter is not clamped by the filter holder. After filtering the hot sugar solution, use I500 mL of wash water instead of the 1 000 ml stated above. **Final Washing of the Membrane Filter** 1. Carefully remove the membrane or the membrane with its pre-filter from the filter holder and place it/them on the wet mesh of the sieve for 1 h. **Drying and Weighing of the Membrane** 1. After the final washing, return the membrane or the membrane with its pre-filter to its/their original petri dish. 2. Dry the dish with the lid removed in the oven for 1 h at 60 to 65º C. **3.** Replace the lid and cool the dish for 30 min in a desiccator. 4. Re-weigh the membrane to the nearest 0.1 mg. 5. For poor filtering sugars, dry the membrane and pre-filter for 1.5 h. 6. The effectiveness of the final washing is essential to the accuracy of the test. This may be checked by spraying occasional membranes, after use, with the l-naphthol/phosphoric acid chromatographic spray reagent and heating to 105°C. The membrane should be entirely free of any trace of violet coloration.
Calculation with units of Expression	Insoluble Matter mg/Kg = $\frac{m_2-m_1}{m_0} \times 10^6$ m_1 : mass in g of the membrane filter or mass of membrane + prefilter. m_2: mass in g of filter + insoluble matter or mass of filter+ pre-filter + insoluble matter. m_0: Weight of the Gooch Crucible (it has not been mentioned above anywhere) with sintered filter and insoluble matter.

Inference (Qualitative Analysis)	NA
Reference	IS 15279 : 2003 Sugar and Sugar Products
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Method No.	FSSAI 04C.051:2024	**Revision No. & Date**	0.0
Scope	Icing Sugar		
Caution	Concentrated Hydrochloric acid is corrosive, causes burns. Wear mask and gloves during analysis		
Principle	Starch is converted to glucose using acid hydrolysis and estimated using a factor		
Apparatus	General Apparatus and Glassware 1. Weighing balance 2. Heating system 3. Filtration system 4. Boiling water bath 5. Reflux Condenser		
Chemicals	1. Icing Sugar. 2. Distilled water. 3. Alcohol. 4. Whatman No. 1filter paper. 5. Hydrochloric acid. 6. Molisch Reagent. 7. Sodium hydroxide.		
Preparation of Test Samples	NA		
Extraction/Procedure	1. Weigh suitable quantity of sample. 2. Dissolve with 100 mL of hot water. 3. Cool, add equal volume of alcohol. 4. Stir and let stand for 2 h. 5. Filter the solution through Whatman No. 1 filter paper or equivalent. 6. Wash the precipitate with 50%, v/v alcohol until the washings does not answer the Molisch test for sugars. 7. Transfer the precipitate with 200 mL hot water into a flask. 8. Add 20 mL HCl and connect the reflux condenser. 9. Heat in boiling water bath for 2.5 h. 10. Cool, neutralise with NaOH and dilute to 500 mL. 11. Determine % of glucose by Lane Eynon's method as given in 6.4.1		
Calculation	Starch % = Glucose % x 0.90		
Inference (Qualitative Analysis)	NA		
Reference	A.O.A.C 21st edn, Official Method of Analysis(2019) Method no.925.50, Starch in Confectionery.		
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Method No.	FSSAI 04C.052:2024	**Revision No. & Date**	0.0
Scope	Cane sugar and refined sugar, Khandsari Sugar		
Caution	Wear Gloves while handling chemicals		
Principle	Excess Calcium Oxide is estimated (as amount of Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) ions both) using EDTA. EDTA forms a complex with Ca^{2+}&Mg^{2+} ions.		
Apparatus/Instrument	General Apparatus and Glassware 1. Calibrated Brix spindle 2. Brix Cylinder 3. Conical flasks - 250 mL 4. Beakers – 100 and 200 mL 5. Funnels 6. Pipettes- calibrated at 10 mL		
Materials and Reagents	1. Ethyl, di-amino tetra acetic acid (EDTA) 2. Ammonia Liquor 3. Lead Sub acetate 4. Potassium Ferrocyanide powder 5. Potassium iodide 6. Eriochrome Black T 7. Rectified spirit or absolute alcohol		
Preparation of Reagents	1. ~~(~~EDTA~~)~~ solution – Weigh accurately 6.6473 g EDTA into a beaker, dissolve in distilled water and make up the volume to 1000 mL. 2. Eriochrome Black T –Weigh0.1 g Eriochrome Black T in a 100 mL volumetric flask and dissolve the same in rectified spirit or absolute alcohol. Make up to the volume and use as indicator.		
Sample Preparation	Grind if necessary and mix to a uniform mass. Thoroughly mix the sample in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container		
Method of analysis	1. Make a 15 °Brix solution of the sample. 2. Transfer about 150 mL of the solution to a conical flask. 3. Clarify the solution with Lead acetate.		

	4. Transfer about 60 mL of the clarified solution to a dry conical flask or flask previously rinsed with the clarified solution. 5. Add Potassium Ferrocyanide powder little by little till no further precipitate forms. 6. Shake thoroughly and filter. 7. Test the filtrate with Potassium Iodide. 8. Collect the lead free filtrate in a conical flask. 9. Pipette out 10 mL of lead free filtrate in a clean conical flask previously rinsed with distilled water and dried. 10. Add 5 – 6 drops of ammonia liquor and 4-5 drops of indicator when a pink colour appears. 11. Titrate against EDTA solution shaking the flask after each addition of EDTA solution. The end point is indicated by a sharp change of colour from red to blue. 12. Note down the volume of the titrant.
Calculation with units of expression	Calcium oxide mg / 100 g = V x 100 mg per L of diluted solution.
Inference (Qualitative Analysis)	NA
Reference	I.S.I. Handbook of Food Analysis (Part II) – 1984
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Method No.	FSSAI 04C.053:2024	**Revision No. & Date**	0.0
Scope	Dextrose		
Caution	Wear Gloves while handling chemicals		
Principle	Acidity of the sample is determined by titration with sodium hydroxide to a phenolphthalein indicator end point after thorough gelatinization to free acids		
Apparatus/Instrument	General Apparatus and Glassware 1. Weighing balance. 2. Conical Flask - 250 mL 3. Burette - 50 mL		
Materials and Reagents	1. Dextrose. 2. Phenolphthalein Indicator solution 3. Standard Sodium hydroxide solution		
Preparation of Reagents	• Phenolphthalein Indicator Solution: (0.2 % w/v) Dissolve 0. 2 g of phenolphthalein in 60 mL of rectified spirit and add sufficient quantity of water to produce 100 mL. • Standard Sodium hydroxide Solution - 0.02N		
Sample Preparation	Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Store sample in a tightly stoppered bottle, Withdraw portions for analytical determinations.		
Method of analysis	1. Weigh accurately about 5 g of the material in to a conical flask. 2. Dissolve it in 50 mL of distilled water free from carbon dioxide gas. 2 Titrate the content of the conical flask with standard sodium hydroxide solution using phenolphthalein solution as indicator. Pink colour will be the end point.		
Calculation with units of expression	Acidity, as mL of 0.02 N standard Sodium hydroxide solution required to neutralize ,5 g of Sample = $\frac{250xVN}{M}$ Where,		

	V = volume, in mL, of standard Sodium hydroxide solution required to neutralize the quantity of the material taken for the test, N = normality of standard Sodium hydroxide solution; and M == mass, in g, of the material taken for the test).
Inference **(Qualitative Analysis)**	NA
Reference	IS 874:2011 Dextrose Monohydrate
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Method No.	FSSAI 04C.054:2024	**Revision No. & Date**	0.0
Scope	White Sugar, Refined Sugar, Bura Sugar, Icing Sugar, Misri, Gur Or Jaggery		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion. 2. Wear gloves and face protection while doing analysis.		
Principle	Synthetic acidic colour(s) is dyed on to wool in acidic medium and extracted (stripped) from the wool into aqueous alkaline medium. If the wool is not dyed then report absence of added artificial colouring matter. If the wool is dyed, it indicates the presence of a coal-tar dye. Acidic coal tar dyes are permitted and basic coal tar dyes are non permitted colors.		
Apparatus/Instrument	General Apparatus and Glassware (1) Pipette (2) Beaker (3) Flask. (4) Soxlet extractor. (5) Whatmman No.1 filter paper. (6) Wollenthread.		
Materials and Reagents	1. White knitting wool 2. Petroleum ether 3. Sodium hydroxide 4. Distilled water 5. Ammonia (0.88 sp. gr) 6. Sodium chloride 7. Acetic acid 8. Isobutanol 9. Butanol 10. Phenol		
Preparation of Reagents	(1) White knitting wool: - Extract pure white wool in a soxhlet extractor with petroleum ether for 2-3 h to remove fat. Boil in very dilute solution of sodium hydroxide and then in water to free it from alkali. (2) Paper: Whatman No. 1 chromatographic paper or equivalent. (3) 1 mL (0.88 sp. gr) ammonia + 99 mL water. (4) 2.5% aqueous sodium chloride . (5) 2% sodium chloride in 50%, v/v ethanol. (6) Acetic acid solution in water (1:3). (7) Iso-butanol-ethanol-water (1: 2 : 1, v/v). (8) n-butanol-water-glacial acetic acid (20 : 12 : 5, v/v) . (9) Iso-butanol-ethanol-water (3: 2: 2, v/v): to 99 mL of this add 1mL of (0.88 sp gr.) ammonia.		

	(10) 80 g phenol in 20 g water.
Sample Preparation	**Part A** **Cane sugar and refined sugar, Bura, Gur or Jaggery, Icing sugar, Liquid glucose** Grind if necessary and mix to a uniform mass. Thoroughly mix raw sugar (Gur, Jaggery) in minimum time. Break up any lumps on a glass plate or in a pestle and mortar. Transfer to a dry stoppered container. **Part B** 1. *Preliminary treatment of food*: Assuming that an acidic colour is present, the preliminary treatment involves removing interfering substances and obtaining the dye in acid solution prior to boiling with wool. 2. Non-alcoholic beverages e.g. soft drinks: As most foods in this group are acidic they can be usually treated directly with wool, otherwise, slightly acidify the food with acetic acid. 3. Alcoholic liquids (e.g. Wine): Boil to remove alcohol and acidify if necessary as in (2). 4. Starch based foods (e.g. cakes, custard powder etc): Grind 10 g of sample thoroughly with 50 mL of 2 % ammonia in 70% alcohol, and allow it to stand for an hour and centrifuge. Pour the separated liquid into a dish and evaporate on water bath. Take up the residue in 30 mL dilute aceticacid. 5. Candied fruits: Treat as in (4). 6. Products with high fat content (e.g. Sausages, meat, fish paste): De-fat the sample with light petroleum and extract the colour with hot water (acidify etc. as usual). Note that oil soluble colours tend to give coloured solutions in organic solvents. 7. If the extraction is difficult treat with warm 50-90% acetone or alcohol (which precipitates starch) containing 2% ammonia. The organic solvent should be removed before acidifying as in (4).
Method of analysis	Extraction of the colour from the food: Acidic Dyes 1. Introduce about 20 cm length of woollen thread into a beaker containing about 35 mL of the prepared acidified solution of the sample and boil for a few min till the woollen thread is dyed. 2. Take out the woollen thread and wash it with tap water. 3. Transfer the washed woollen thread to a small beaker containing dilute ammonia and heat again. If the colour is stripped by the alkali, the presence of an acid coal-tar dye is indicated. 4. Remove the woollen thread. Make the liquid slightly acidic and boil with a fresh piece of woollen thread. Continue boiling until the colour is taken by

	the woollen thread. 5. Extract the dye from the woollen thread again with a small volume of dilute ammonia, filter through a small plug of cotton and concentrate the filtrate over a hot water bath. 6. This double stripping technique usually gives a pure colour extract. Natural colours may also dye the wool during the first treatment, but the colour is not usually removed by ammonia. Basic dyes 7. Basic dyes can be extracted by making the food alkaline with ammonia, boiling with wool and then stripping with dilute acetic-acid. 8. At present, all the permitted water soluble coal-tar dyes are acidic, hence an indication of the presence of a basic dye suggests that an unpermitted colour is present.
Calculation with units of expression	NA
Inference (Qualitative Analysis)	If the wool is dyed, it indicates the presence of a coal-tar dye. Presence of basic coal tar dyes indicate the presence of non permitted colors.
Reference	Manual Methods of Analysis for Adulterants and Contaminants in Food, I.C.M.R 1990
Approved by	Scientific Panel on Methods of Sampling and Analysis

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Method No.	FSSAI 04C.055:2024	**Revision No. & Date**	0.0
Scope	Dextrose		
Caution	1. Once sample is opened, seal it in airtight manner after taking test portion 2. Wear gloves and face protection while doing analysis.		
Principle	The principle of the method is based on the ability of glucose to reduce Fehling's solution. The standardized Fehling's solution is then used to determine the amount of glucose in an unknown sample using methylene blue as indicator.		
Apparatus/Instrument	(a) Volumetric Flask:- 500 ml (b) Single mark pipette:- 10 ml (c) Analytical Balance (d) Chronometer (e) Conical Flask with a Narrow Mouth :- 300 ml (f) Burette with stopper or Bent burette:- 50 ml graduated in 0.1 ml and suitable protection plate. (g) Heating Device:- Which would ensure boiling under the condition as indicated in method of analysis and which would enable lighting, to determine the end point without having to move the conical flask.		
Materials and Reagents	**Fehling's solutions** **Solution A:-** Aqueous solution of copper sulphate containing 69.28 g of $CuSO_4.5H_2O$ per litre. **Solution B:-** Aqueous solution containing 346 g of sodium potassium tartrate, potassium tetrahydrate and 100 g of sodium hydroxide per litre. Note:- As the mixed solutions are unstable in the presence of air, mixing is done during the test. **Calibration of Fehling's Solution:-** Titrate the Fehling's solution thus prepared with the standard solution of dextrose (A.1) as indicated in (A.2). Let V_s be the number of millilitres of dextrose solution used; this value should be equal to 40 ± 0.5 ml. Otherwise, the Fehling's solution should be adjusted. A.1:- **Standard Solution of Dextrose**:- Dissolve 2.5 g of the pure dextrose dried beforehand at 70 ^oC under reduced pressure, in distilled water to make 1000 ml.		

	A.2:- **Determination:-** With the help of the pipette, transfer 10 ml each of solution A and B of the Fehling's solution into the 300 ml conical flask. Add a boiling regulator, that is pumice stone or glass marbles and enough of water to raise the total volume of liquid (Fehling's solutions + Water + test solution) to 75 ml ± 5 ml at the end of the titration. In order to determine the quantity of water that is added, it is often necessary to carry out a preliminary determination using the quantity of water, considered effective for covering evaporation, for instance 40 ml. Put the conical flask on heating device. Right from the start of heating, pour with the help of the burette the sugar solution amounting within 0.5 ml of the anticipated end point (determined by a preliminary test in which the test solution is gradually added till the end point is reached). Adjust the heating in such a way as to make the solution boil within 2.75 ± 0.25 min and then make no more adjustment till the end of the test. The boiling should be brisk and continuous all along the operation otherwise air would enter the flask and oxidize its content. It is therefore essential that the flask is not shaken after the heating starts. After 2 minutes of boiling, add 2 drops of methylene blue solution and complete the titration drop by drop. At the approach of the end point, observe a time of ten to fifteen seconds between the addition of two successive drops. Carry out the operation till the blue colour vanishes. Titration should be completed within 1.5 to 2.0 min after the addition of the indicator. **Methylene Blue Indicator:-** One percent aqueous solution.
Sample Preparation	Weigh to the nearest 0.001 g a quantity of sample in such a way that after dilution to 500 ml, the test solution contains approximately 0.25 g of reducing sugars expressed as dextrose for 100 ml (This test portion generally weighs between 1 and 10 g).
Method of analysis	**Procedure** 1) With the help of the pipette, transfer 10 ml each of solution A and B of the Fehling's solution into the 300 ml conical flask. 2) Add a boiling regulator, that is pumice stone or glass marbles and enough of water to raise the total volume of liquid (Fehling's solutions + Water + test solution) to 75 ml ± 5 ml at the end of the titration. 3) In order to determine the quantity of water that is added, it is often necessary to carry out a preliminary determination using the quantity of

	water, considered effective for covering evaporation, for instance 40 ml. 4) Put the conical flask on heating device. Right from the start of heating, pour with the help of the burette the sugar solution amounting within 0.5 ml of the anticipated end point (determined by a preliminary test in which the test solution is gradually added till the end point is reached). 5) Adjust the heating in such a way as to make the solution boil within 2.75 ± 0.25 min and then make no more adjustment till the end of the test. The boiling should be brisk and continuous all along the operation otherwise air would enter the flask and oxidize its content. It is therefore essential that the flask is not shaken after the heating starts. 6) After 2 minutes of boiling, add 2 drops of methylene blue solution and complete the titration drop by drop. 7) At the approach of the end point, observe a time of ten to fifteen seconds between the addition of two successive drops. Carry out the operation till the blue colour vanishes. 8) Titration should be completed within 1.5 to 2.0 min after the addition of the indicator.
Calculation with units of expression	Let V_1 be the number of millimeters of test solution used. If this volume is not 40 ± 5 ml, it is necessary to modify the concentration of the test solution. It is recommended to check at the end of the titration if the final volume falls within the prescribed limits. Carry out two determinations on the same sample. The reducing sugar expressed as dextrose, percent by mass, is equal to : $(2.5/1000) \times (V_s) \times (500/V_1) \times (100/E) = (125\ V_s) / (V_1 \times E)$ Where E = mass, in g, of the test portion V_s = Volume, in ml, of the standard dextrose solution (A.1) used for the calibration of the Fehling's solution and V_1 = Volume, in ml, of the test solution required for reducing 20 ml of the Fehling's solution.
Inference (Qualitative Analysis)	NA
Reference	IS: 1S 873:1974 Specification For Liquid Glucose
Approved by	Scientific Panel on Methods of Sampling and Analysis